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The Journal of Laboratory and Clinical Medicine

VOL. 24 NOVEMBER, 1938 NO. 2

CLINICAL AND EXPERIMENTAL

PLETHYSMOGRAPHIC STUDIES WITH SPECIAL REFERENCE TO WAVES OF RESPIRATION*

S. J. MARTIN, M.D., F. S. MARCELLUS, AND P. SYKOWSKI, B.A., ALBANY, N. Y.

THE effect of respiration on blood pressure in the larger arteries was investigated many years ago by Lewis[1] and Erlanger and Festerling.[2] Further studies of vascular phenomena have been facilitated greatly by the use of the plethysmograph, and recently emphasis has been placed upon the peripheral portion of the vascular tree. Johnson[3] described a sensitive glass tube finger plethysmograph in which the volume changes were recorded by photographing the movements of a drop of colored alcohol in a horizontal calibrated tube attached to the plethysmograph. From these photographic records it was possible to estimate a 0.002 c.c. change in volume. In subsequent studies[4, 5] reference was made to superimposed waves associated with respiration which were believed to be due to (a) periodic vasomotor changes, (b) changes in cardiac output, or (c) movement of the animal. Bolton, Carmichael, and Stürup[6] used an optical system of flat mirrors to record photographically the vascular changes in the finger, but gave no special significance to waves synchronous with respiratory movements. Turner[7] described a new sensitive apparatus consisting of a pulse recorder, optical capsule, and photokymograph to study vascular phenomena in the terminal phalangeal region. The appearance of waves of respiration was not mentioned.

Using a new and sensitive apparatus in the study of peripheral vascular phenomena, waves of respiration were recorded along with pulse and volume changes. An attempt in this investigation was made to calibrate this apparatus

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and also to determine the underlying factors responsible for the appearance of the respiratory waves. A preliminary report of this study has appeared.[8]

PROCEDURE AND RESULTS

The apparatus used for this study was constructed by the General Electric Co., Schenectady, N. Y. It is a photoelectric recorder which combines all the advantages of direct acting recorders with those of the most sensitive instruments. Plethysmographic changes are transmitted by simple rubber tubing to the basic element or tambour mounted on the apparatus. Any motion of the tambour in turn moves a small mirror which, when in equilibrium, casts a beam of light exactly between two photoelectric cells. With the aid of an optical system of mirrors, imbalance of the central beam activates the right or left photoelectric cell. This procedure accordingly moves a siphon type recording pen which follows an indicating element and records on a continuous strip chart (Fig. 1). The apparatus is simple to manipulate and affords one a direct record and without recourse to photographic procedures. The records can be readily reprinted.

1. *Calibration.*—Preliminary studies in peripheral vascular phenomena revealed the extreme sensitivity of this new type of recording device. An attempt was, therefore, made to calibrate it. A circular pyrex glass chamber (9.0 by 2.5 cm.) was constructed with three outlets, the center one of which was attached to a microcapillary pipette graduated to 0.001 c.c. One outlet was connected to the recording device and the other served for introduction of a colored fluid. A 1 c.c. tuberculin syringe was attached to the pipette to facilitate withdrawal of fluid from the glass chamber. Pinch clamps were placed at one outlet and between the pipette and chamber. Beginning with the colored fluid in the pipette at zero, increments of 0.001 c.c. were introduced into the chamber and the magnitude of deflection of the pointer noted. This procedure was continued until a maximal deflection was noted and then the deflections were measured upon withdrawing 0.001 c.c. increments.

It was found that the magnitude and accuracy of the deflections, when the tambour of the apparatus was fixed at zero, depended upon the internal diameter and not upon the length of the tube connecting the chamber and photoelectric recorder. Rubber tubes of nine different lengths and of four different diameters were used. The most satisfactory tube for animal work had an internal diameter of 0.15 cm. and an outside diameter of 0.4 cm. The length could be variable up to 175 cm. With such a tube, the siphon pen of the apparatus showed a deflection to either side of the midline of 48 mm. representing an increase in volume in the chamber of 0.16 c.c. Therefore, 1 mm. deflection was equivalent to a change of 0.003 c.c. in volume. Changes of 0.001 c.c. could be detected, but only the 0.003 c.c. increment could be accepted as accurate.

Although this apparatus requires little technical knowledge to manipulate it, it is exceptionally sensitive and accurate. The only significant precaution in its use is to avoid extraneous vibrations or undue movement of the animal. The calibration, in particular, has served to form a basis for quantitative study of peripheral vascular phenomena in man.

2. *Factors Concerned With Waves of Respiration in Plethysmographic Studies.*—In determining the role played by nervous and mechanical factors in the appearance of respiratory waves, plethysmographic records were made of the fingers and toes of 3 men and 3 monkeys, and of the hind paw of 3 rabbits,

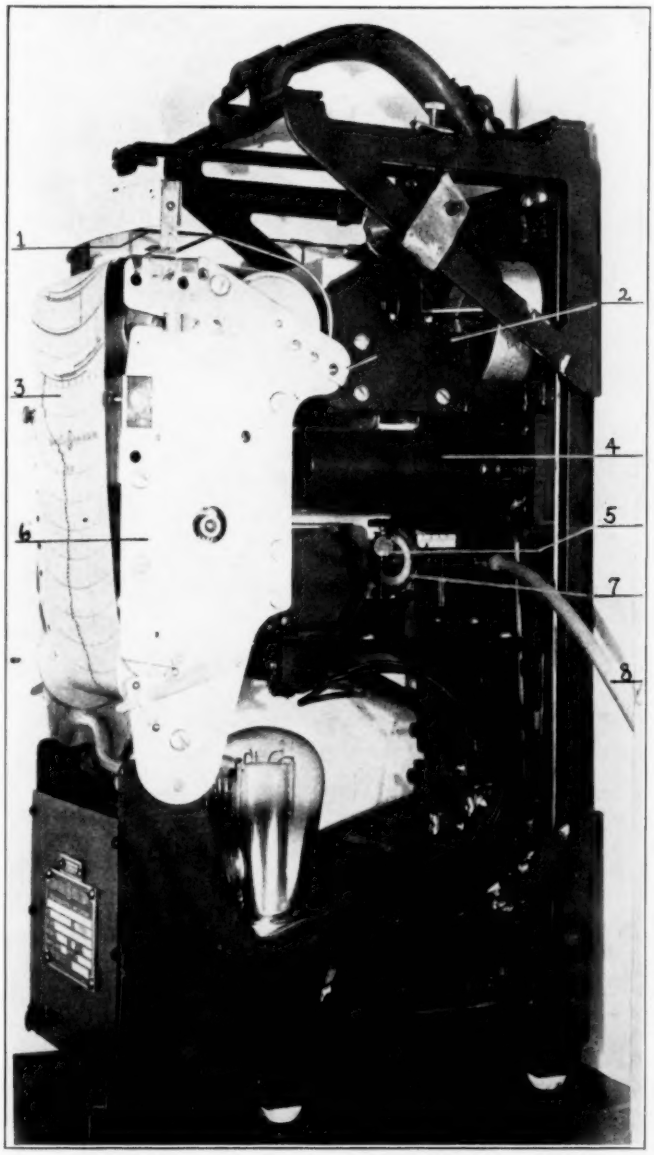


Fig. 1.—The photoelectric recorder used in plethysmographic studies of digits of man and of the paw of animals. *1*, siphon pen; *2*, indicating element; *3*, strip chart; *4*, photoelectric cell; *5*, mirror; *6*, chart carriage; *7*, basic element or tambour; *8*, rubber tube to plethysmograph. (Through the courtesy of the General Electric Co., Schenectady, N. Y.)

5 rats, 5 guinea pigs, 5 cats, and 22 dogs. The plethysmographs were made of pyrex glass tubing, one end of which was connected to the photoelectric recorder and the other end snugly attached with a synthetic nonelastic rubber

dam and a layer of vaseline at the second interphalangeal region, generally of the finger, or at the first interphalangeal region of the toe. In animals, the plethysmograph was attached 1 to 5 cm. proximal to the pad of the hind paw. Air leakage was eliminated before experimentation.

In addition to the pulse and volume changes, waves synchronous with respiration could be noted in all observations made, except in the case of guinea pigs and rats and in a number of instances in the toes of man and monkey

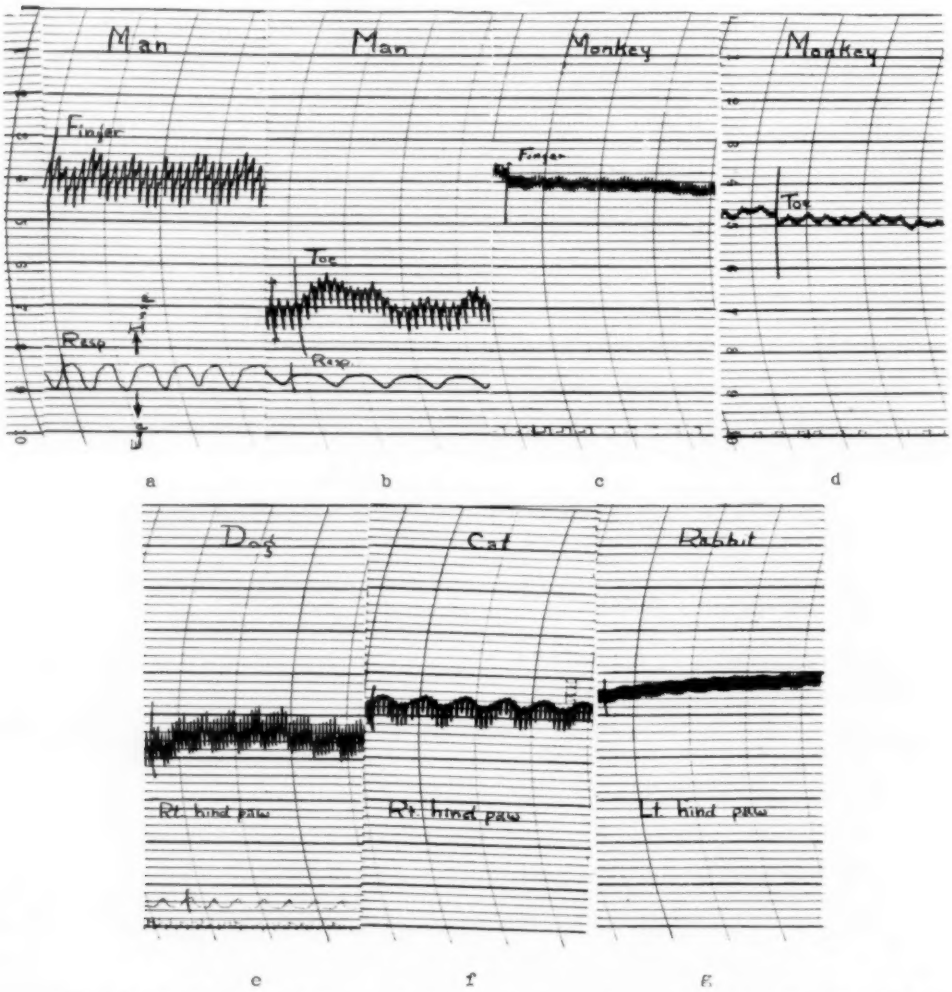


Fig. 2.—Plethysmographic records taken on a continuous strip by the photoelectric recorder of the digits of man and monkey and of the hind paw of animals. Note the pulse rate and waves synchronous with respiration. The bottom tracing in *a*, *b*, and *e* represents respiratory chest movements. Decrease in volume of the digits is noted by movement of the pointer toward the zero mark; increase in volume or vasodilation, by movement toward the ten mark. Line 5 represents the normal or initial volume. The vertical lines indicate ten-second intervals. Waves synchronous with respiration may be seen in records of all animals.

(Fig. 2). When these respiratory waves were present, a definite correlation could be established between plethysmographic volume and phases of respiration. Decrease in volume followed inspiration and increase in volume generally was seen after expiration. It was significant to note that the magnitude of the

respiratory waves in the plethysymograph tracing was directly proportional in all cases to the increase in plethysmographic volume. This correlation was greatly magnified in cats and dogs anesthetized and receiving endotracheal artificial respiration. In these animals, if the inspiratory phase was purposely prolonged, a decrease in the plethysmographic volume of the paw was noted and the respiratory waves disappeared. However, in the sustained phase of expiration, the result was variable in that either vasoconstriction or slight or marked vasodilation occurred (Fig. 3).

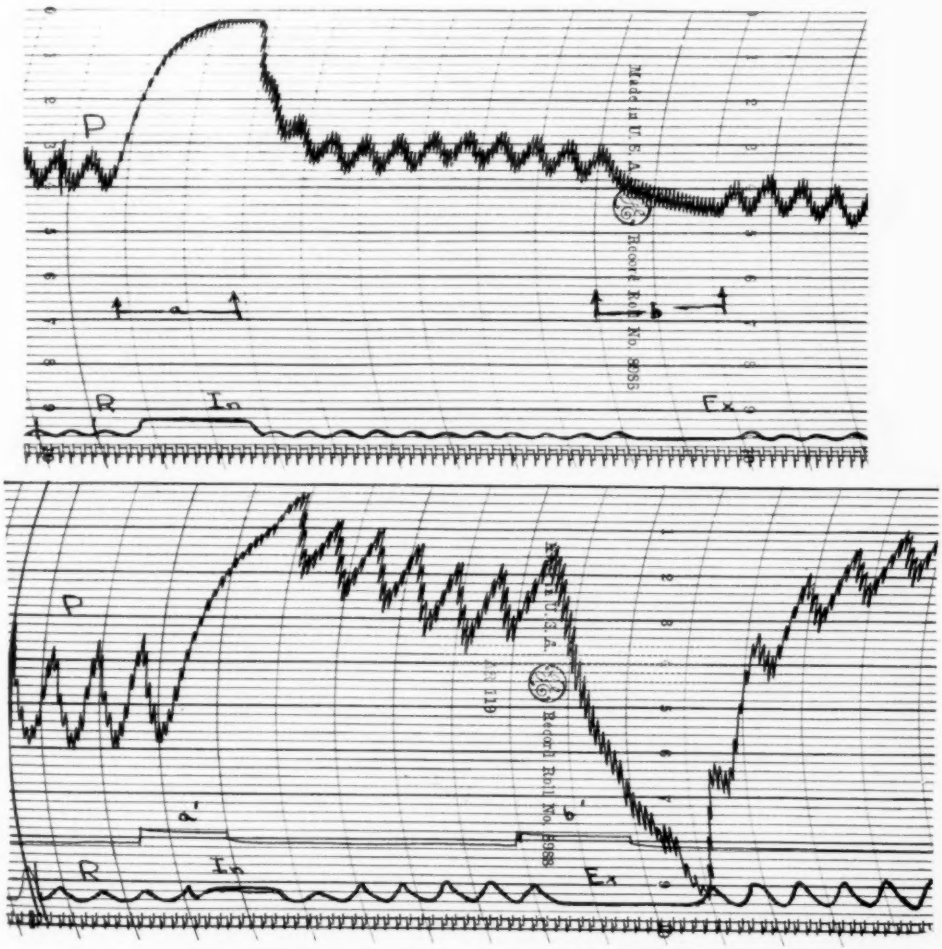
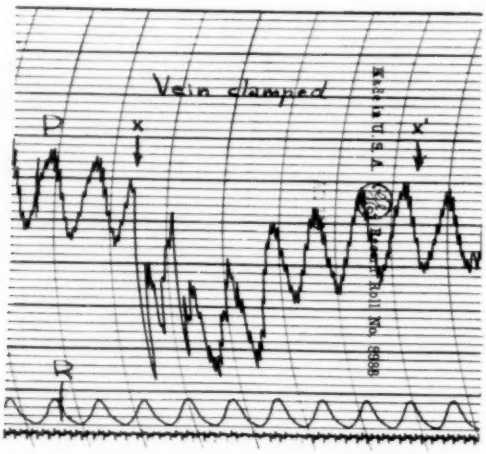


Fig. 3.—Plethysmographic records (*P*) of the hind paw of dogs receiving endotracheal artificial respiration (*R*). The upper record shows vasoconstriction during sustained inspiration (*In*). Slight vasodilation is seen during expiration (*Ex*). In the lower record, marked vasodilation is noted during the expiratory phase (*Ex*).

a. *Mechanical Factor.*—The importance of the mechanical factor responsible for the appearance of the waves of respiration was revealed in the following experiments. The glass plethysmograph was attached to the right or left hind paw of 5 cats and 9 dogs and under nembutal anesthesia, the corresponding femoral nerve and femoral and iliac vessels were exposed. This surgical procedure resulted in a transient reflex increase in heart rate, a vasoconstriction

of the vessels of the paw followed by a vasodilation. The changes in the amplitude of the respiratory waves were secondary to the degree of vasoconstriction noted.

No change was noted in character of the respiratory waves, except a slight increase in magnitude due to the vasodilation in the paw, after temporary and light clamping of the femoral or common iliac veins. When the femoral artery



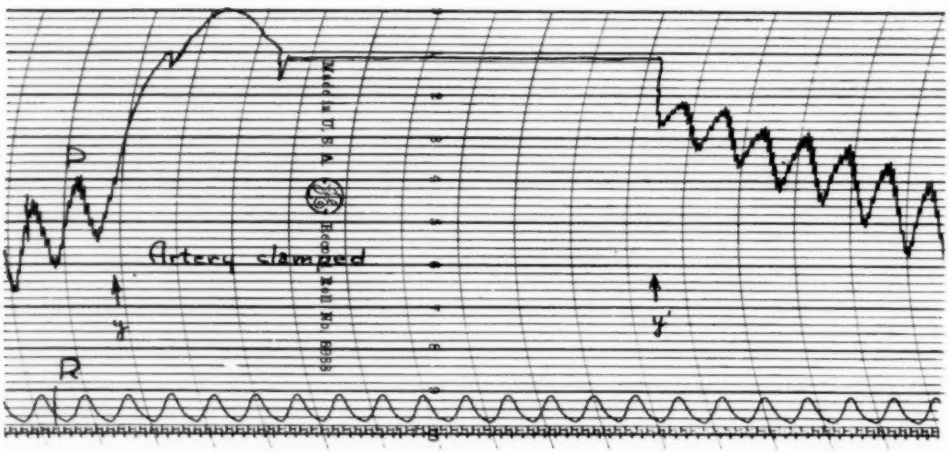


Fig. 4.—Plethysmographic records (P) of the hind paw of a dog receiving endotracheal respiration (R). Upper record: marked vasodilation upon temporary clamping (x-x') of the femoral vein with increase in amplitude of respiratory waves. Lower record: no respiratory waves during the sudden decrease in blood volume produced by temporary clamping (y-y') of the femoral artery. Return of the waves of respiration to normal height can be noted in both records upon release of clamps.

was occluded with fingers, of course, the pulse disappeared along with the respiratory waves despite the intact innervation (Fig. 4). In 3 of the dogs, the vessels were painted with 70 per cent alcohol solution and in 3 others the connective tissue coat for 3 to 4 cm. was stripped in order to eliminate the influence of the sympathetic fibers. No change was noted as compared to the

above results when the different vessels were clamped. It would seem, therefore, that the appearance of the waves of respiration was due to mechanical causes rather than to a nervous influence.

In another series of 5 dogs, plethysmographic records were taken of the hind paw after the leg was severed from the body at the inguinal region, except for the femoral artery and vein. Temporary clamping of the vein again

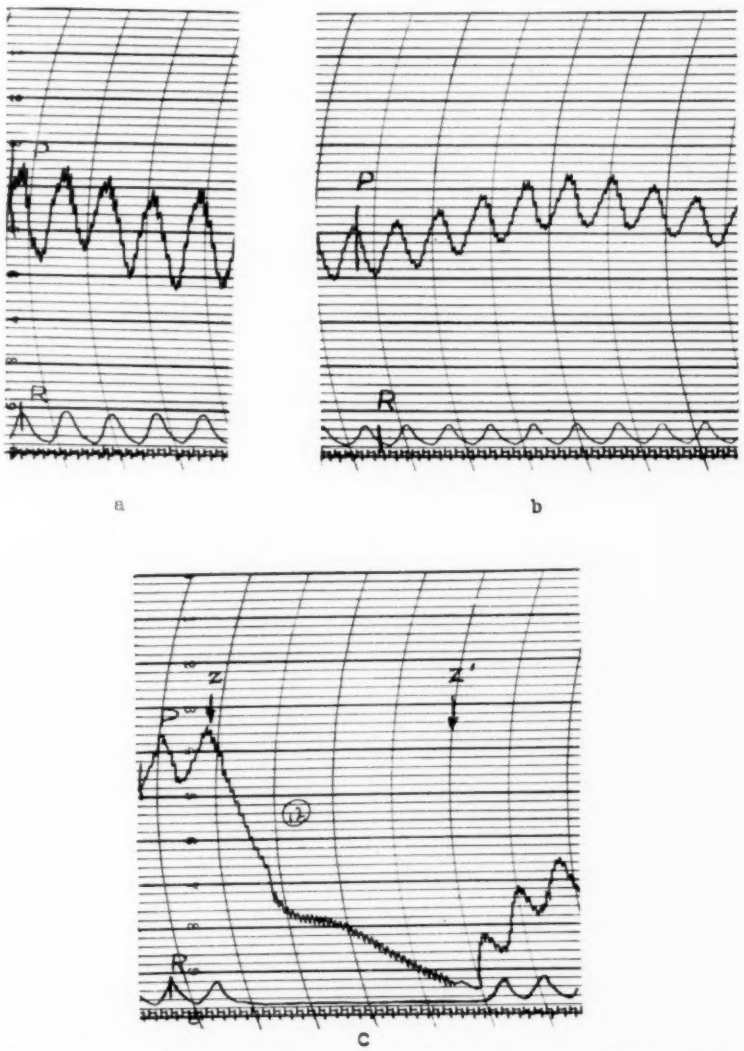


Fig. 5.—*a*. Normal plethysmographic tracing of hind paw of a dog under endotracheal artificial respiration. *b*. After a glass cannula was inserted between proximal and distal cut ends of the femoral artery when the leg was severed from the body, except for the vessels. Note the decrease in amplitude of the respiratory waves. *c*. Absence of respiratory waves and increase in blood volume of the paw upon temporary cessation of respiration (z-z').

did not influence the appearance of the respiratory waves, while occlusion of the artery did. To rule out all possible nervous impulses coming from the body, a glass cannula was inserted between the proximal and distal cut ends of the femoral artery in these dogs. The magnitude of the respiratory waves

was diminished about 33 per cent, but the waves were nevertheless clearly evident (Fig. 5a, b). This decrease in size may have been due (1) to removal of the nervous factor, or (2) to clot formation in the cannula with resultant diminished blood flow to the paw. The respiratory waves in these cannulated preparations were magnified by increased thoracic excursions and promptly disappeared when the artery was pinched off, or when the tracheal artificial respiration was temporarily stopped (Fig. 5c). In 3 of these animals receiving artificial respiration, the diaphragm was resected and the abdomen closed. Such a procedure did not eliminate the appearance of the respiratory waves. However, when the chest and abdomen were subsequently opened, the respiratory waves immediately disappeared despite the fact that the heart was beating and the lungs were expanding under artificial respiration. The remaining 2 dogs were sacrificed by an intravenous injection of pentobarbital, and the artificial respiration was continued after the cessation of the heart beat.

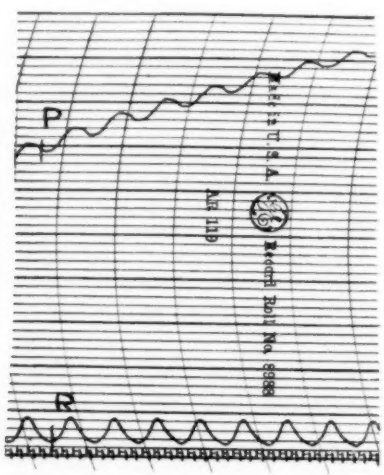


Fig. 6.—Plethysmographic record of hind paw of a recently dead and pulseless dog still receiving artificial respiration. Waves synchronous with respiration are still present.

Plethysmographic records of the paw in these recently dead and pulseless dogs still receiving artificial respiration revealed the presence of respiratory waves, although to a markedly diminished extent (Fig. 6).

It was concluded, therefore, that the appearance of superimposed waves of respiration in plethysmographic records of the paw are primarily caused by the mechanical factor due to the movement of the thoracic cage upon the blood flow. The magnitude of these waves appears dependent upon the degree of arterial relaxation in the paw.

b. *Nervous Factor.*—Because of the possibility of a nervous influence in the decrease of the amplitude of the respiratory waves in the cannula experiments, referred to above, it was considered advisable to investigate this factor more thoroughly. Accordingly, plethysmographic records of the hind paw of 8 additional dogs were studied in the following experiments. Under nembutal anesthesia, the femoral and iliac vessels and nerves were exposed, and the nerves cut. No effect was noted on the waves of respiration. In 4 of these animals, a

transection of the spinal cord at the twelfth thoracic and first lumbar region was performed. Similarly, the respiratory waves remained unchanged.

Mild to moderate faradic stimulation was applied to the central and peripheral cut ends of the femoral and sciatic nerves and the peripheral region of the transected cord. In all instances, the magnitude of these waves was dependent upon primary reflex changes in the size of the vessels. Thus, with each vasoconstriction in the paw the waves decreased in size, and conversely, upon vasodilation they increased. In no case were they absent. When endotracheal artificial respiration was temporarily stopped, faradic stimulation to the cut nerves or spinal cord did not cause the waves to reappear. Thus, it may be concluded that the somatic or spinal nerves exert no influence on the appearance of the waves of respiration.

In 3 of the 4 dogs with a cord transection and section of the femoral and sciatic nerves, a moderate faradic stimulus was applied to the exposed femoral artery for the purpose of stimulating the sympathetic nerves. The waves of respiration detected in the arteriolar bed of the paw did not disappear, although their magnitude was decreased due to the slight vasoconstriction produced. Thus, the sympathetic innervation is not primarily concerned with the appearance of the respiratory waves.

DISCUSSION

The influence of thoracic movements on blood pressure is well known and can be readily noted in recording the pressure of aortic, carotid, and femoral arteries and in plethysmographic studies of the spleen and kidney. In regions where arterioles predominate, such as the digits of man and the paw of animals, little attention has been paid to waves synchronous with respiration. These waves have been detected with the use of the photoelectric recorder in the peripheral portion of the vascular tree. Our results indicate that they are mechanical in origin due to the increased pressure exerted on the thoracic and abdominal arterial blood by respiratory movements. The use of the sensitive photoelectric recorder has enabled us to detect such minute increases in pressure of the blood in the arteriolar bed of the paw. That they may be due to changes in cardiac output has not been entirely ruled out. Their prompt disappearance when artificial respiration is temporarily stopped or when the chest or abdomen is opened may depend in part upon decreased cardiac filling.

Our data also show that no direct nervous factor is involved in the transmission of the respiratory pulsations. However, upon inspiration there is a peripheral vasoconstriction which, according to Goltz[9] and Bolton, Carmichael, and Stürup,[6] is of reflex origin. These vasomotor impulses to the arterioles of the paw secondarily influence the magnitude of the respiratory waves. Thus, with vasoconstriction, the waves of respiration decrease in amplitude. Extraneous factors, such as movement of the animal or vibrations of the animal board, have been ruled out by the use of long rubber tubing connecting the plethysmograph and the photoelectric recorder placed on different tables.

This investigation does not confirm the suggestion made by Johnson[4] that the appearance of waves of respiration in plethysmographic studies of arteriolar regions is due to vasomotor action or to body movements of the animal.

Since the magnitude of the waves of respiration depends upon the patency of the arteriolar bed of the vascular system, their detection may be of clinical value. Thus, by determining the amplitude of the respiratory waves in response to the use of suitable vasomotor stimuli, one can differentiate between mechanical and nervous factors in the etiology of peripheral vascular disease. Such a procedure could be of prognostic as well as of diagnostic importance. Further, the relative stability of the vasomotor system and deviations from the normal response to certain arbitrary stimuli may be determined by noting the changes in the size and character of the respiratory waves in plethysmographic studies of digits.

The use of our plethysmograph and photoelectric recorder, aside from the detection of the waves of respiration, has served to record pulse rate and volume changes in digits. This procedure can be utilized in the study of peripheral vascular responses to vasomotor drugs, anesthetics, and to varying degrees of surgical trauma. The apparatus possibly may be used to show pressure and volume changes in arteries, uteri, or intestinal loops.

SUMMARY

A sensitive photoelectric recorder was calibrated and used in plethysmographic studies of digits of man and of the paw of animals. The apparatus was easy to manipulate and afforded one a direct record without recourse to photographic procedures. In addition to pulse rate and volume changes, waves synchronous with respiration were recorded. These waves were found to be mechanical in origin and essentially due to increased pressure with each respiration of the thoracic and abdominal blood, a pressure transmitted to the paw. Nervous factors have been ruled out. The magnitude of the respiratory waves, however, depends upon the degree of arteriolar relaxation. It is suggested that the appearance and character of these waves may be of importance in the study of peripheral vascular phenomena in man.

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NEUTROPENIA FOLLOWING SULFANILAMIDE*

Report of a Case

Herbert W. Jones, M.D., and C. Phillip Miller, M.D., Chicago, Ill.

IT WAS to be expected that the current increase in the use of sulfanilamide would give rise to occasional instances of toxicity. The occurrence of fever and skin rashes as toxic manifestations has been noted[1] and three cases of acute hemolytic anemia have been reported by Harvey and Janeway,[2] another by Kohn,[3] and one case of severe and two of moderate anemia by Jennings and Southwell-Sander.[4] In addition to these, six instances of malignant neutropenia have been recorded in this country and England. Only one of the four recovered.

LITERATURE

The first report of agranulocytosis attributable to sulfanilamide is by Plumer.[5] A woman of 54, suffering from subacute endocarditis (streptococcus viridans) of about five months' duration was given 1 gm. of sulfanilamide a day for four days, and 1.3 gm. for thirty-one days, when it was discontinued because of nausea and vomiting. Three days thereafter the white count had fallen to 400, with no neutrophiles; it rose the next day to 1,600, but extensive gangrenous infection of the mouth and pharynx had already developed and death occurred on that day.

Young[6] reports agranulocytosis with fatal outcome in a man of 53 years, suffering from acute rheumatism, who had taken 3 gm. of sulfanilamide for eighteen days. Skin rashes (not described) are mentioned as occurring on four days, the first of which antedated the beginning of sulfanilamide administration. Model's[7] case was a man of 20, suffering from a recurrence of rheumatic fever. Three grams of sulfanilamide were given for eighteen days and then discontinued. Two days later the leucocyte count was found to be 600. In spite of blood transfusion and pentnucleotide, death occurred the following day. Jennings and Southwell-Sander[4] observed agranulocytosis in a 39-year-old woman who suffered from ulcerative colitis. Sulfanilamide, 4.5 gm. per day, had been given for three weeks and discontinued because her symptoms had disappeared. Two days later evidence of agranulocytosis began and increased in severity for four days, when the total white blood count was less than 400, none of which were polymorphonuclear cells. The administration of pentnucleotide was followed immediately by a rapid rise in the polymorphonuclear count and general improvement and recovery.

*From the Department of Medicine and the Kuppenheimer Foundation of the University of Chicago.

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Quite recently two additional cases have been added. The patient of Schwartz, Garvin, and Koletsky[8] was a man of 32, treated for a penile ulcer with sulfanilamide over a period of twenty-one days, during which time he took a total of 56.6 gm. The typical symptoms of agranulocytosis developed and resulted in death. Berg and Holtzman[9] report a fatal attack of agranulocytosis in a man of 22 years, who took 38 gm. of sulfanilamide in eleven days for acute gonorrhea.

The gravity of neutropenia as a toxic manifestation of a drug so widely used as sulfanilamide prompts the present authors to add another case report to the literature, for it is only by the gradual accumulation of such data that the administration of this valuable drug can be safeguarded.

CASE REPORT

The patient, N, a native-born white man, 26 years old, an unemployed salesman, was admitted to the University of Chicago Clinics, November 5, 1937, suffering from acute gonococcic anterior urethritis (his first attack), which had begun the day before, five days after

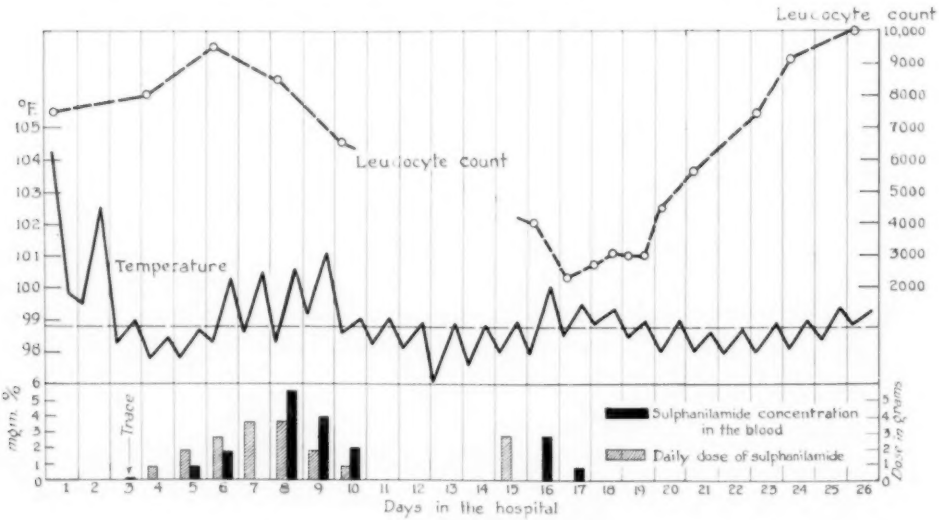

Fig. 1.

exposure. He was treated in the Urological Out-Patient Department, where 0.6 gm. sulfanilamide was ordered to be taken four times a day. At subsequent visits on November 8 and 11 his discharge was found to contain gonococci, and sulfanilamide therapy was continued. On November 15 he reported that he had been having headache, backache, and fever for three days, during which period he had taken very little food or fluid. His urethral discharge had ceased. The urine was clear, containing only an occasional shred and occasional white blood cell. His temperature was 104.4° F.

He was transferred to the medical service and admitted to the Albert Merritt Billings Hospital. Chief complaints were headache, backache, and pains in his legs, especially the knees. Positive findings on physical examination were temperature, 104.2° F., dryness of the tongue and skin, distinct odor of acetone in the breath (although none could be demonstrated in the urine), moderate injection of the pharynx, and a soft blowing systolic murmur heard over the third interspace, just to the left of the sternum.

The temperature curve and leucocyte counts are given in the accompanying chart as well as the sulfanilamide content of the blood as determined by Marshall's method.[10] Blood cultures made on each of the first three days remained sterile, and cultures of the nose,

throat, and stools developed only the customary inhabitants of the upper respiratory and intestinal tracts. Urinalyses were uniformly negative, except for leucocytes and occasional red cells. Blood Wassermann and Kahn were negative.

As certain of the toxic rashes described seemed to have been brought out by sunlight, one treatment with ultraviolet light was given over the left arm. Only a mild erythema resulted.

TOTAL AND DIFFERENT LEUCOCYTE COUNTS*

DAY	TOTAL	POLYMORPHONUCLEAR NEUTROPHILES	BASOPHILES	LYMPHOCYTES	MONONUCLEARS
16	4,100	25	5	69	1
17	2,300	5	3	77	12
18	2,600	9		52	39
18	3,100	5		64	24
19	3,000	15	2	59	21
20	4,500	25	3	57	13
21	5,600	26	1	49	11
23	7,400	41	3	50	5
24	9,100	57	1	30	10
26	10,000	64	1	32	3

*For most of these counts the authors are indebted to Miss Alice Plain.

The patient's symptoms subsided with his fever, and the heart sounds became clear, but on the fourth day the urethral discharge returned and contained numerous gonococci. Sulfanilamide therapy was cautiously resumed, beginning with 0.9 gm. and increasing by that amount each day until a daily dose of 3.6 gm. was reached. His temperature rose again, and on the second day of maximum dosage the patient complained of mild dull headache, chilliness, general malaise, and the feeling "that he was coming down with a cold." The drug was discontinued and his fever and symptoms disappeared. During this period his leucocyte count remained normal.

The urethral discharge, which had lessened under full doses of sulfanilamide, once more increased, and on the fifteenth day 2.7 gm. of the drug were administered. The next morning the urethral discharge, which had been examined daily, was found to contain few healthy looking polymorphonuclear cells, and the blood count showed only 4,100 leucocytes, of which 25 per cent were polymorphonuclears. Later that day the patient complained again of headache and chilliness, general malaise, and the feeling "that he was coming down with a cold." On the following day the leucocyte count was 2,300, neutrophiles only 5 per cent. Yellow bone marrow concentrated,* 80 drops a day, was given and continued for six days. The leucocyte count began to rise the next day (the eighteenth), and continued to do so. His symptoms improved and disappeared entirely on the nineteenth day. By the twentieth day the urethral discharge, which had been scant and serous since the sixteenth day, became more profuse and purulent. At no time were gonococci absent. The patient was discharged from the hospital December 18, 1937, and transferred back to the Urological Clinic for ambulatory treatment of his urethritis, from which he had not yet recovered.

COMMENT

Although some doubt was entertained at the time of this patient's admission to the hospital that he was suffering from a febrile reaction to sulfanilamide, subsequent events seem to have established this diagnosis with certainty. The sulfanilamide determination on the blood drawn the first day was unreliable, so its content at the height of his symptoms is unknown. But by the third day, when his blood showed only a trace of sulfanilamide, the patient was symptom free; and after the drug had been increased again to its former dosage, the fever and some of his symptoms once more returned.

*Supplied by Armour and Company, Chicago.

During this second period of intoxication, when he was suffering from mild headache, general malaise, and the feeling "that he was coming down with a cold," the concentration of sulfanilamide (free plus "combined") in the blood was only 5.6 mg. per cent, less than one might expect from the dose he had taken, probably because of the high level at which fluid intake and output was maintained. Marshall and his co-workers[11] have emphasized the rapidity of sulfanilamide excretion by the kidneys.

The same symptoms recurred for the third time when the blood contained only 2.8 mg. per cent of sulfanilamide, and this time were accompanied by severe neutropenia. It seems highly probable, therefore, that this patient was becoming increasingly susceptible to the drug.

It must be borne in mind that this patient had taken 2.4 gm. of sulfanilamide a day for ten days before his admission to the hospital. In this connection it will be recalled that in the reported cases of agranulocytosis cited in this paper, the drug had been administered over periods varying from eleven to thirty-five days.

One is led to the conclusion that vulnerability to the toxic action of sulfanilamide developed about the beginning of the second week of its administration and increased during the period of observation here reported. Accumulation of the drug in the body seems to have been ruled out by the estimations of its concentration in the blood. Hageman and Blake,[1] who observed fever, sometimes accompanied by skin rash on the tenth to twelfth day, were unsuccessful in their effort to prove it an allergic phenomenon.

SUMMARY

A patient who had taken sulfanilamide for eight days developed fever and generalized aching pains. Two attempts to resume therapy were followed by a return of symptoms, the last time accompanied by severe neutropenia. Determinations of its concentration in the blood indicate increasing susceptibility to the drug.

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A NOTE ON THE DIAGNOSIS OF HYPERTENSIVE CARDIOVASCULAR DISEASE WITHOUT HYPERTENSION*

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THE middle-aged cardiopath who presents moderate to marked cardiac enlargement, an absence of significant valvular lesions, and normal or low blood pressure, with or without signs of heart failure, constitutes an interesting diagnostic problem from an etiologic viewpoint. According to present-day cardiologic teaching, the diagnoses of "chronic myocarditis" and "chronic nonvalvular disease of the heart," usually given to cases of this type, are no longer acceptable.

Coronary artery disease forms the background for this clinical syndrome in certain instances, although in these, cardiac hypertrophy of great degree is an unusual finding. Other inciting factors in a small number of cases are chronic adhesive mediastinopericarditis, syphilitic coronary ostial disease, and latent or masked hyperthyroidism. The large majority of such cases, however, as has been emphasized by O'Hare and his associates,[1] are probably instances of hypertensive cardiovascular disease in which the blood pressure has fallen to a normal, or in some cases a subnormal, level.

The variability of the blood pressure in essential hypertension is well known. Ayman[2] reported the finding of normal blood pressures in 56 per cent of 76 cases of established essential hypertension, some of which exhibited widespread vascular changes. Such spontaneous remissions in blood pressure are as a rule transitory and of relatively short duration. With the advent of heart failure of the congestive type in hypertensive cases, marked reductions in blood pressure to normal or subnormal levels are not uncommonly encountered and are frequently persistent. In some instances, with the restoration of cardiac compensation, the blood pressure returns to its previous high level. In establishing an etiologic diagnosis in these cases of hypertensive cardiovascular disease without hypertension seen for the first time, O'Hare[1] has emphasized the importance of an antecedent history of hypertension, and the presence of retinal arteriosclerosis. The former, however, while of great importance, is frequently lacking. Additional confirmatory findings would appear to be the presence of a diffuse fibrous type of peripheral arteriosclerosis, arteriolar sclerosis determined by muscle biopsy and, perhaps, evidence of impairment of renal function.

The important contributions of Hines and Brown[3] concerning the blood pressure response to their standard vasomotor stimulus, the cold pressor test, have revealed that 98 per cent of patients with essential hypertension give abnormal maximal responses. From these findings they conclude that patients

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with essential hypertension possess hyperreactive vasomotor mechanism. These observers further referred to "a group of healthy subjects in the later decades of life who had hyperreactions and changes in the retinal arterioles which were indicative of essential hypertension, but who had normal or subnormal levels of (resting) blood pressure." It occurred to us that in suspected hypertensive cardiopaths presenting normal or low blood pressures, such hypersensitivity of the vasomotor apparatus to stimulation might perhaps be retained, even though the "resting" blood pressures were no longer elevated. If so, the procuring of a maximal, or "hypertensive type" of response to the standard stimulus of such a case would furnish important confirmatory evidence toward establishing an accurate etiologic diagnosis. During the prosecution of our studies on the cold pressor test we have made certain observations which tend to show that in the majority of instances such appears to be the case.

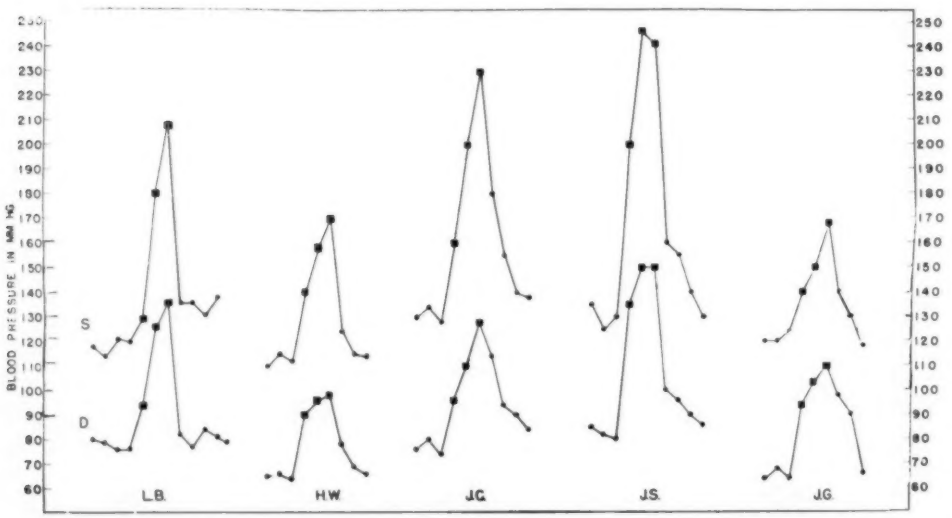

Chart 1.—Blood pressure response to the cold pressor test in five subjects with hypertensive cardiovascular disease with normal blood pressures. An excessive or "hypertensive" type of response was obtained in each instance. The squares represent readings taken during the application of the stimulus.

Seven subjects with known hypertensive cardiovascular disease were encountered in whom the blood pressures had fallen to normal or subnormal levels. These patients had been under observation for from two to ten years, during which time there had been persistent and marked elevation of both the systolic and diastolic pressures. In addition, all showed cardiac enlargement as well as retinal and peripheral arteriosclerosis. All were males, their ages ranging from 35 to 55 years. The apparent cause of the blood pressure reduction, in five cases, was congestive heart failure; in one case, coronary artery closure with cardiac infarction; and in the other case, a streptococcus throat infection.

We subjected all seven of these patients to the cold pressor test. The vasomotor stimulus employed was that of Hines and Brown,[3] with certain minor modifications.[4] Blood pressure readings were taken at intervals of thirty, ninety, and one hundred and fifty seconds during the period of stimulus

(cold) application, instead of the two readings at thirty and sixty seconds as in the original method.

Five of the seven subjects gave a decided maximal or "hypertensive type" response, according to our criteria (Chart 1). Of the remaining two, one gave an essentially normal response, and the other a borderline type of reaction. In the latter instance it was felt that a primary nephritis might possibly have been the etiologic factor of the hyperpiesia.

From these results the conclusion seems justified that in many patients with hypertensive heart disease in whom the blood pressure has fallen to a normal or subnormal level, the hyperirritability of the vasomotor apparatus is retained and vasomotor stimulation will evoke an exaggerated blood pressure rise similar to that observed in the usual hypertensive subject *with* hypertension. It would seem, therefore, that the exhibition of such a response in a cardiac patient of the type described would provide a valid reason for believing that a pre-existing hypertension was the important etiologic factor in the cardiac pathology presented.

SUMMARY

Seven patients with established hypertensive cardiovascular disease whose blood pressure had fallen to normal or subnormal levels were subjected to the cold pressor test and the blood pressure response noted. In five instances the typical hypertensive type of response was obtained. This fact suggests that the application of such a procedure to patients of this type would materially aid in the clarification of the etiologic cardiac diagnosis.

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ON THE NATURE OF THE ANTIPERNICIOUS ANEMIA PRINCIPLE IV*

Search for Nitrogenous Bases Isolation of Choline

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THE response of a patient with pernicious anemia to a mixture of potato juice and tyrosine was described in an earlier paper.[1] The "red substance" of Raper was suspected to be concerned with the beneficial effect observed. Further clinical tests with (1) dihydroxyphenylalanine, (2) dihydroxyphenylalanine oxidized with peroxide and traces of iron salts, and (3) dihydroxyphenylalanine oxidized by oxygen in alkaline solution have shown, through uniformly negative results, that this idea was probably erroneous.

Because, however, a reticulocyte response had been observed after giving potato juice and tyrosine, it seemed justifiable to examine potent preparations for the presence of some of the simpler nitrogenous bases known to occur in potatoes and other plants.[2] The group of bases including choline and the betaines (betaine, trigonelline, stachydrine, etc.) was selected for study because they, like the active principle in liver extract, form precipitates with phosphotungstic acid.[3]

A preliminary test of liver extract with potassium periodide (Stanek) and with potassium bismuth iodide (Kraut) established the presence of much material precipitable with these reagents. If liver extract is first treated with lead acetate and the excess of lead removed, these "alkaloidal reagents" often yield crystalline precipitates. Although these precipitates are formed readily, they are not in themselves suitable for the positive identification of specific substances. Therefore, the following adaptation of customary procedures was undertaken.

METHOD

One hundred grams of liver extract (Lilly, for oral use) was dissolved in 1 liter of water. Lead acetate in excess of that needed for complete precipitation was added, and the precipitate filtered off and discarded. The filtrate was treated with hydrogen sulfide to remove the excess of lead. The lead sulfide was washed once by shaking up with 1 liter of water and filtering. The combined filtrates were evaporated under reduced pressure to a volume of 1 liter. Then for each 100 c.c. of liquid, 17.5 c.c. of concentrated hydrochloric acid (sp. gr. 1.180) was added, and the mixture autoclaved for one to one and one-half hours at 60 pounds pressure. (This treatment destroys possible interfering substances.[4, 5]) After the liquid has cooled somewhat it was filtered and then cleared by boiling repeatedly with animal charcoal and filtering. (The animal charcoal is first thoroughly extracted with boiling water.) The clear, almost colorless liquid was evaporated to a thick syrup

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under reduced pressure. The syrup was repeatedly extracted with hot 95 per cent alcohol, leaving a residue of inorganic matter. The hot alcoholic extracts were filtered through a hot water funnel and then treated with an excess of mercuric chloride in alcoholic solution. A voluminous white precipitate formed at once and was filtered off after standing twenty-four hours. It was washed with a little alcoholic bichloride solution, then suspended in 2 liters of hot water and treated with hydrogen sulfide to saturation. The mercuric sulfide was filtered off and washed with water. The combined filtrates were evaporated to a syrup under reduced pressure and again extracted with hot 95 per cent alcohol. The alcoholic solution was evaporated to a thick, light amber gum. The gum was taken up in a little water, in which it is very soluble, and treated with an equal volume of concentrated hydrochloric acid. This mixture was poured into a concentrated solution of gold chloride in 3 N hydrochloric acid. A voluminous bright yellow precipitate formed at once. It was recrystallized from its mother liquor by warming, then filtered off, washed with a little water, and dried in the oven at 105° C. After cooling in a desiccator, it weighed 2.4 gm. and melted to a red liquid with the evolution of gas at 253° C. with moderately rapid heating. This gold salt was recrystallized four times from dilute hydrochloric acid containing a little gold chloride. The melting point remained the same. This material was then analyzed for its elements. There were found: C 13.60 per cent, H 3.07 per cent, N 3.02 per cent, and Au 44.39 per cent. These values correspond well with the formula: $C_5H_{14}NOCl.AuCl_3$, the chloroaurate of choline, for which is calculated: C 13.54 per cent, H 3.16 per cent, N 3.16 per cent, and Au 44.47 per cent. Further recrystallizations of the gold salt from water and from dilute hydrochloric acid did not change the melting point, if the same rate of heating was observed in all tests.

A sample of choline hydrochloride (Pfanstiehl) was converted into its chloroaurate, which was then recrystallized twice from dilute hydrochloric acid containing a little gold chlorine. The manner of crystal formation and the crystals were like those in the experiments with the material from liver.

The mixed melting point determination was made as follows: A sample of the aurate from liver and a sample of the aurate from choline were dried in the oven and cooled in the desiccator at the same time. One capillary tube was used for each salt alone, and one for an intimate mixture of the two. All tubes were of the same size and thickness and filled to the same depth and degree of packing. All three were then attached to the same thermometer and heated in the same bath together. The behavior was identical for all three: with slow heating, softening and reddening began at 248° C., and melting was complete at 250 ° C. All formed red liquids with the evolution of gas.*

COMMENT

Although the presence of choline in liver was demonstrated many years ago,[6] it was surprising to find it representing the nitrogenous bases in liver

*These precautions are necessary because the melting point of choline chloroaurate has been given at anywhere from 238° to 256° C. It seems to vary with the mode of preparation of the salt, the thickness and packing in the tubes, and the rate of heating, etc.

extracts which are prepared by methods that should have separated it because of its solubility in both water and alcohol. The rather remote possibility of an effect on pernicious anemia is being investigated.

SUMMARY

A study of liver extract for its content of nitrogenous bases showed the presence of at least 1 per cent of choline.

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STUDIES ON THE MECHANISM OF LEUCOCYTOSIS

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THESE experiments were designed to test two of the possible causes of leucocytosis: withdrawal of leucocytes from the blood stream or the presence of an irritant in the blood. The first of these was tested by the withdrawal of leucocytes by hemorrhage and by their withdrawal into an abscess area. The second idea was attacked through the use of substances which can be readily followed in the tissue where they were injected and in the blood.

EXPERIMENTS

The usual blood techniques (lateral ear vein blood) were employed; cover slip smears were stained with Wright's stain. Chinchilla rabbits with a normal blood picture were used throughout. Total and differential counts were done for two days previous to as well as directly before any experimental procedure. Following the initiation of the experiment counts were done at two- to four-hour intervals for twenty-four hours, eight to twelve hours after.

Withdrawal Experiments.—1. Ten animals comprised this group. After establishing their blood cytologic picture as normal, quantities of blood ranging

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from 4 to 14 c.c. were removed by venepuncture from the femoral vein, care being exercised not to traumatize tissues. No animal showed a leucocytosis, even though as many as 140 million leucocytes were removed with 14 c.c. of blood (the count being 10 thousand).

2. In inflammation leucocytes are withdrawn from the circulation into the inflammatory area. In six animals abscesses were produced by the intracutaneous injection of streptococci. White counts were then made at the usual intervals. After each count, the injected area from one animal was removed for sectioning. Caution was used in having the abscess sectioned as nearly as possible through its exact center. The leucocytes in such a section were counted by starting at one edge of the abscess and progressing in parallel fields. The volume of the tissue in the section was then determined. Considering the abscess to be lenticular in shape, which is usually the case, the total number of leucocytes in the whole abscess was fairly accurately determined. Using this method, the total number of leucocytes in the abscesses was found to be:

	Time After Streptococcus Injection	
	2 Hours	24 Hours
Leucocytes in abscess	938,000	122 million
Leucocytosis increase per cent above normal	30	150

At twenty-four hours the total circulating leucocytes [with a leucocytosis of 25 thousand (average for this time) and an animal weighing 2,000 gm.] number some 5 billion. It is difficult to believe that 122 million leucocytes in an abscess could cause over 5 billion to appear in the circulation, so that withdrawal, either by venepuncture, which gives no leucocytosis, or into an abscess, cannot account for leucocytosis.

These experiments point to the presence of some substance in the blood which brings about leucocytosis.

Experiments to Test for the Presence of the Injected Substance.—1. Silver nitrate. Fourteen animals were used. Four control animals were injected intracutaneously with 2.5 c.c. of distilled water; they remained normal. The other animals were injected with 2.5 c.c. of a 10 per cent silver nitrate solution. Almost immediately edema and reddening appeared at the injection site. This reaction progressed during the first twenty-four hours. By the seventy-second hour necrosis appeared in the center. This spread and by the fourth day was as large as 2 by 2.5 cm. The blood picture of these animals changed rapidly. They showed a leucopenia within four to eight hours after injection. In one animal the drop was from its normal of 9,000 to 2,300. Following primary leucopenia, which lasted twenty-four to forty-eight hours, there was a marked leucocytosis. Counts as high as 19,000 were common. This lasted five to ten days. The leucopenia and leucocytosis were due to variations in polymorphonuclear leucocytes. Chart 1 shows a typical curve.

Silver determinations were done on the femoral vein blood, femoral bone marrow, sternal bone marrow, spleen, liver, lungs, and skin. The tissue or blood was obtained at two, eight, fourteen, and thirty-six hours; four-, seven-, and nine-day intervals. The blood or organ is ashed and treated with hydro-

chloric acid. After digestion with zinc and filtering, the residue is treated with nitric acid. The addition of sodium chloride solution gives a positive test of abundant white precipitate (silver chloride) provided silver is present. This method is sensitive to one part in 800,000 (Benedict[1]). No positive tests were obtained on either the blood or any internal organ. Skin sections from the injected areas invariably showed large quantities of silver.

2. Egg albumen. Twelve animals were used, three as controls which remained normal. Nine animals were injected with sterile crystalline egg albumen intracutaneously, with 4.0 c.c. doses, at four-day intervals, five injections being given to each animal. (The egg albumen was prepared freshly by the addition of ammonium sulfate and recrystallization. The salt was dialyzed off and the solution sterilized with a Berkefeld filter. Micro-Kjeldahl determinations for nitrogen placed the protein content at 4.5 per cent.) Following each of the sensitizing doses, a leucopenia set in within

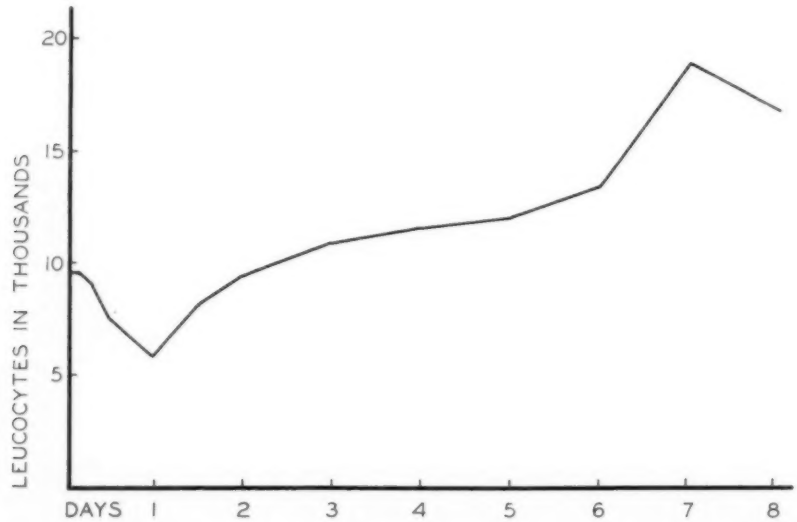

Chart 1.—This chart shows the leucocyte curve obtained after the intracutaneous injection of 2.5 c.c. of sterile silver nitrate. Note the primary leucopenia and the relatively late leucocytosis. This is a typical curve.

two hours and lasted twelve hours. The lowest count so obtained was 3,800; this animal's normal was 9,400 just prior to injection. The leucopenia was less with each successive dose. The urine was analyzed for albumin during these injections and was found to be present in abundance.

Following the last sensitizing dose, fourteen days were allowed to elapse. Into a new skin area, 2 c.c. of albumen were then injected. This injection caused a typical Arthus reaction. Reddening and edema set in early, and necrosis appeared about the fourteenth hour. The necrosis spread up to the seventy-second hour. The leucocyte study during the production of the lesion is shown in Chart 2. A severe primary leucopenia (polymorphonuclear cell loss) set in within two hours, reaching its lowest by the sixth hour. In between twelve and twenty-four hours the cell count returned to normal, to increase to 200 per cent or more above normal by the seventy-second hour.

This leucocytosis lasted three to five days. The highest count obtained was 47,000. By the third day there was bone marrow hyperplasia. The leucocytosis was caused by an increase in polymorphonuclear cells. The data below show the averages, in thousands, for the animals.

Directly Before Injection		Injection		Following Injection		
	10.1			2 hr. 5.6	4 hr. 2.5	
7 hr.	10 hr.	24 hr.	48 hr.	72 hr.	96 hr.	120 hr.
4.5	4.7	10.5	21.1	21.0	17.6	15.1

After the necrosing injection, the urine of these animals was tested for albumin; none could be found in the highly sensitized animals' urine.

In summary, no silver nitrate or egg albumen could be detected in the blood during leucocytosis. With these two substances, peripheral necrosis is apparently necessary for the production of leucocytosis.

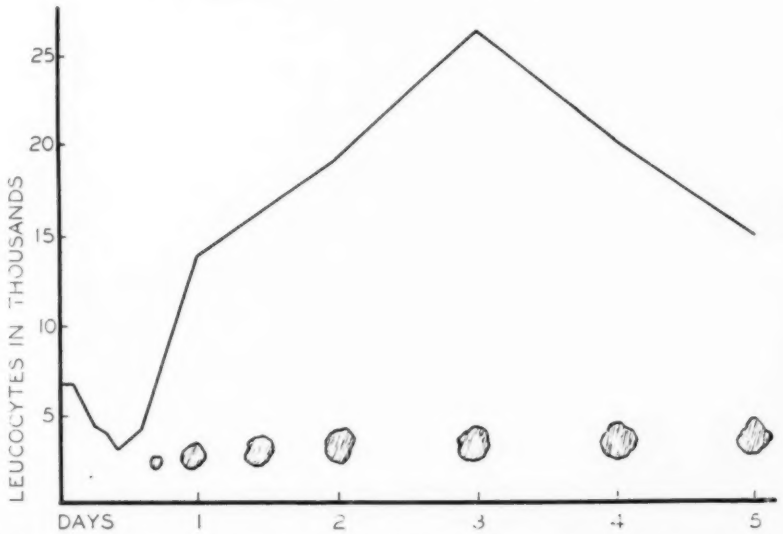

Chart 2.—This chart shows a typical leucocyte curve obtained after injection of the necrotizing dose of egg albumen. The stippled areas at the bottom show the gross necrosis. Notice that the first appearance of this is just at the time the leucocytosis sets in.

DISCUSSION

It is a common clinical observation that postoperatively or following massive hemorrhage, a leucocytosis occurs. However, in such observations there are always factors other than the hemorrhage itself to be considered, such as anesthesia, infection, trauma. Experimentally, Dawson[2] produced leucocytosis by massive hemorrhage; however, he introduced other factors. With caution 14 c.c. of blood were removed from a rabbit without producing a leucocytosis, and recently as much as 30 to 40 c.c. of blood has been taken from a single animal without causing a leucocytosis (Boyd et al.[3]). This is comparable to a massive hemorrhage in man.

As we have seen, even though 122 million leucocytes go from the blood stream into an abscess in twenty-four hours, the circulating leucocytes are 5 billion. This enormous increase is all out of proportion to what would be

expected if leucocytosis were produced by simple withdrawal. Even though the method is an approximation, the disproportion is still striking. In human beings, too, one is struck by the relatively small number of leucocytes, say in a furuncle, compared to the numbers such a lesion will call forth into the blood stream.

These observations suggest that there is some substance in the blood stream which brings about leucocytosis. The present experiments tested for the necrotizing substance itself (silver nitrate and egg albumen). None of either substance could be detected during leucocytosis. The criticism may be offered that small quantities of albumin may escape from the site of injection. In this regard Opie[4] remarks, speaking of antigen (egg albumen), "The same substances introduced in the immune animal are fixed at the site of entry and are not found in the blood." Even more against the idea that it is the albumin as such acting on the bone marrow to produce leucocytosis, is the fact that the leucocyte increase occurs parallel in time to the necrosis of peripheral tissue, and is in proportion to that necrosis. It may be that the inciting agents themselves do diffuse from their site of entry to cause leucocytosis. However, this could not be demonstrated with the agents used. The rapidity with which leucocytosis set in is rather against the idea. The present observations point to a correlation between the advance of necrosis and leucocytosis.

CONCLUSIONS

1. The withdrawal of leucocytes, either by venepuncture or into an abscess area, cannot account for leucocytosis.

2. In acute experiments silver nitrate injected intracutaneously cannot be found in the blood or internal organs. It is recoverable in large amounts from the area of injection.

3. Silver nitrate injected intracutaneously causes necrosis accompanied by a primary leucopenia followed by marked polymorphonuclear leucocytosis.

4. Egg albumen injected into non-sensitized animals diffuses from the injection site and causes a leucopenia.

5. Injected into sensitized animals, egg albumen does not diffuse into the blood stream. Peripheral necrosis occurs with a concurrent well-defined leucocytosis.

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ACHLORHYDRIA IN LEUCEMIA*

OVID O. MEYER, M.D., MADISON, WIS.

IN 1932 Strandell and Torgersruud[1] reported a series of 22 cases of leucemia in which they found gastric achlorhydria to be of frequent occurrence. Particularly was this true in lymphocytic leucemia. These authors reported finding achlorhydria in 7 of 14 cases of myeloid leucemia and in 6 of 8 cases of lymphoid leucemia, and they wondered whether or not achylia was not responsible for gastric complaints which are so common in leucemia. The average age for the group with myeloid leucemia and achlorhydria was slightly younger, but the patients with lymphoid leucemia and achlorhydria averaged more than 10 years older than those with free acid.

All of the gastric analyses in their series were done after administration of an Ewald meal. The stimulus to secretion afforded by the administration of histamine was not utilized. Bloomfield and Polland[2] emphasize that achlorhydria cannot be accepted as definitely existing unless repeated samplings after histamine administration prove negative for free hydrochloric acid.

Since about the time the report of Strandell and Torgersruud[1] appeared, gastric analyses have been made on nearly all of the cases of chronic leucemia admitted to the medical service of the State of Wisconsin General Hospital. These cases, together with the few in which gastric analyses had incidentally been made previous to 1933, are included. If free acid was not demonstrated by a single aspiration after an Ewald meal, 1 mg. of histamine was administered subcutaneously to all cases.

RESULTS

Chronic Myeloid Leucemia.—Gastric analysis was done in 21 cases. Of these only 3, or 13 per cent, of the cases that received histamine, showed absolute achlorhydria. Seven other cases showed an absence of free acid after Ewald meal, but in both of the 2 cases of this group that later received histamine, free acid was found. It is interesting that of the 3 cases with histamine achlorhydria, 1 was a youth of 16. The ages of the other 2 patients were 43 and 56. The average age of the entire group of 21 patients was 44 years.

Chronic Lymphoid Leucemia.—Gastric analysis was done in 19 cases. Of these 10, or 53 per cent, had achlorhydria after histamine administration. Four others had achlorhydria after Ewald meal alone, but when histamine was administered, 3 of these 4 demonstrated the ability to secrete hydrochloric acid. Of the 10 truly achlorhydric subjects, 1 was 38 years old, but the remainder ranged in age from 55 to 75 years. The average age of the group of 19 patients was just under 60 years.

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Aleucemic Leucemia.—The diagnosis in this group was established in most instances by lymph node or bone marrow biopsy. There were 10 cases. Only 3 patients had achlorhydria, and each of these had received histamine. The ages were 53, 55, and 62 years, respectively. The average age of the group of 10 patients was 51.5 years.

Acute Leucemia.—Only 5 cases were available in this group, 4 of lymphoblastic and 1 of myeloblastic leucemia, and consequently the figures can mean little. However, 2 of the cases, both lymphoblastic, showed achlorhydria after histamine. These patients were 49 and 64 years of age.

COMMENTS

Each of these series of cases is small, but they show, in some degree, the trend. Cognizance of the age periods for each group must be taken for as Bloomfield and Polland[2] and Vanzant and others[3] have shown, achlorhydria is increasingly frequent with greater ages. This fact must be recognized, particularly in the group with chronic lymphoid leucemia, where the average age is 60 years. In this group there is a high incidence of achlorhydria, 53 per cent. At this age period one would expect, in healthy individuals, an incidence of achlorhydria of approximately 25 per cent. Consequently, in these cases of chronic lymphoid leucemia, the frequency of achlorhydria appears to be significant.

In the patients with myeloid leucemia, a younger group, an incidence of achlorhydria of 13 per cent is not significant for at the average age of 44 years, this or a greater frequency might be anticipated in any group of individuals. In one case, a youth of 16, persistent achlorhydria was found. The significance of this, however, was not demonstrated as post-mortem examination was not permitted and, therefore, gastric disease was not excluded. The frequency of achlorhydria in the aleucemic leucemia group was 33 per cent. If the series comprised more than 10 cases, the results might be significant, but under existing circumstances, with an average age of 51.5 years, it cannot be so considered, and the suggestion is gained that the incidence of achlorhydria is not unusual. The smallness of the group with acute leucemia likewise permits no significant deduction relative to the incidence of achlorhydria.

It is noted that the incidence of achlorhydria in both myeloid and lymphoid leucemia is distinctly less than reported by Strandell and Torgersruud.[1] This can be explained by their failure to use histamine. Confirmation for this stand is offered by the several observations reported here where free acid appeared only after histamine administration.

In several cases of all types repeated study of the gastric contents, months apart, was done, but in all instances the results were consistently positive or negative for the respective cases.

It was not possible to demonstrate any relationship between the level of the leucocytes or erythrocytes and achlorhydria.

SUMMARY AND CONCLUSIONS

Gastric analysis after histamine administration was done in patients with leucemia. The incidence of achlorhydria in the various types of leucemia was as follows: Chronic myeloid leucemia 13 per cent, chronic lymphoid leucemia

53 per cent, aleucemic leucemia 33 per cent. The average ages of the groups was 44, 60, and 51.5 years in the order named. Two of 5 patients with acute leucemia had achlorhydria.

The incidence of achlorhydria is not significant in chronic myeloid leucemia nor, probably, in aleucemic leucemia. The frequency of achlorhydria in chronic lymphoid leucemia apparently is significant even though this disease occurs in an older age group. The explanation for this finding in chronic lymphoid leucemia is not as yet available.

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THE ADSORPTION OF PHENOL BY PROTEIN IN PREPARATION OF CERTAIN BIOLOGIC PRODUCTS*

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INTRODUCTION

THE refining and concentration of antiserums and antiplasmas involve in general terms the precipitation of different protein fractions by the addition of salts, by suitable dilution with water at definite hydrogen ion concentrations, and by other methods. In the processing of these materials in order, if possible, to maintain sterility, or at least to reduce bacterial growth to a minimum, preservatives are generally added. Results will be presented which show that when phenol is used for this purpose, the protein precipitates may carry down or adsorb considerable amounts of phenol. This factor must be considered before the addition of phenol to the final product in order to avoid excessive amounts of this preservative.

EXPERIMENTAL METHODS

Phenol Estimations.†—"The method of analysis consisted in the quantitative formation of tribromphenol by the addition of a bromide-bromate solution and titration of the excess bromine with thiosulphate after the addition of potassium iodide, hydrochloric acid, and starch. The method was used first by Koppeschaar,[2] studied further by a number of workers, and a careful reinvestigation was given in detail by Scott[3] several years ago.

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†The following description is taken from the paper by McGuire and Falk.[1]

"In view of Scott's extended study and detailed presentation of the method, it will not be repeated here. For the removal of protein, the well-known Folin-Wu precipitation was used. To 5 c.c. of the material, 35 c.c. of water (or saline) were added, then 5 c.c. of 10 per cent sodium tungstate solution and 5 c.c. of 0.67 N sulphuric acid. The mixture was shaken, allowed to stand at room temperature for thirty minutes, and filtered through paper. To 40 c.c. of the filtrate, 160 c.c. water were added, and the phenol estimation carried out.

"Duplicate determinations as a rule agreed within 0.5 per cent of the amount of phenol found. Using the purest phenol obtainable, satisfactory recoveries, within 2 per cent, of the amount added, were obtained."

Refining and Concentrating Antiserums.—The precipitation of proteins in the refining of the antiserums as carried out in this Laboratory follows essentially the procedure described by Klein, Greenwald, and Falk,[4] with certain modifications to be described in detail in another connection. The following main points are to be considered in their bearing on the present problem. The designation of the successive steps follows the description given in the former paper.

b. *Dialysis of Serum.*—A definite concentration of phenol was added to the original serum. A considerable part of this phenol content was removed from the serum as a result of dialysis. At the conclusion of the dialysis, a certain amount of precipitate was present.

c. *"Acid Protein" Precipitation.*—The dialysate, after the addition of sodium chloride to N/20 and 0.3 per cent phenol, was brought to pH 5.1. The precipitate was removed by centrifuging.

e. *Precipitation of Antibacterial Substance.*—The liquid was brought to the desired pH (as determined in preliminary tests; either 5.9, 6.1, or 6.8), four volumes of distilled water containing 0.2 per cent phenol added. After standing overnight in the cold, the precipitate was removed by centrifuging.

f. *Solution of Antibacterial Substance.*—The precipitate was dissolved in the requisite amount of solvent containing sodium chloride and phenol.

Phenol estimations were carried out at the following stages:

b. Original serum; serum after dialysis; centrifuged precipitate; supernatant.
c. Centrifuged acid precipitate; supernatant.
e. Centrifuged antibacterial precipitate; supernatant.
f. Final product.

The solid contents, dried at 110° C., of the precipitates in b, c, and e, were determined, and the amount of phenol adsorbed by the protein calculated. This calculation includes correcting for the supernatant liquid and its phenol, protein, and sodium chloride contents present in the moist centrifuged precipitates.

When phenol was to be added directly to antibacterial preparations, it was mixed with ether in the proportion of three parts of the former to four parts of the latter.

Experimental Results.—The phenol contents are given as weights per cent per 100 ml. of liquid or precipitate. For example, 0.3 per cent phenol signifies

0.3 gm. phenol per 100 ml. of liquid or precipitate (volume of precipitate as measured in calibrated centrifuge tubes).

The following typical experiments are presented:

Experiment 1. Antipneumococcus (Type II) Preparation

	Per cent Phenol
Original serum (1040 ml.)	0.262
Mixture after dialysis (1140 ml.); after sampling (1020 ml.) centrifuged	0.094
Supernatant (938 ml.)	0.092
Precipitate moist (82 ml.)	0.120
Precipitate dried (14 gm.)	0.256
Sodium chloride and phenol (approx. 0.3 per cent) added to mixture.	
Mixture before acid protein precipitation (1010 ml.)	0.380
Acid protein precipitation—pH 5.1 (volume loss due to manipulations)	
Supernatant (900 ml.)	0.378
Precipitate acid protein moist (20 ml.)	0.488
Precipitate acid protein dried (1.6 gm.)	1.750
Supernatant—pH 5.9—4 volumes distilled water containing 0.2 per cent phenol	
Supernatant (4400 ml.)	0.240
Precipitate—antibacterial protein moist (68 ml.)	0.344
Precipitate—antibacterial protein dried (15.4 gm.)	0.697

The antibacterial moist precipitate after centrifuging was 68 ml. in volume and 0.344 per cent in phenol content, containing, therefore, 0.234 gm. of phenol. The volume of the original serum was 1,040 ml. If the final volume of the preparation were to be one-sixth of the initial volume, or 173 ml., the moist precipitate would be diluted to that volume with salt solution of suitable concentration. In order to obtain the proper phenol concentration, a calculated amount would be added. If the final volume were to be 173 ml. as just indicated, and if 0.5 per cent phenol concentration were desired, 0.865 gm. would be added. Actually then, because of the phenol present in the moist precipitate, the phenol content would be 0.635 per cent (instead of the desired 0.5 per cent). If 0.35 per cent phenol concentration were desired in the final product, 0.606 gm. would be added, but this would give a concentration of 0.486 per cent. If the final volume of the preparation were to be one-twelfth of the initial volume, or 87 ml., the amount of phenol calculated to give 0.5 per cent would give an actual concentration of 0.769 per cent, and the amount calculated for 0.35 per cent an actual concentration of 0.620 per cent.

Experiment 2. Antipneumococcus (Type II) Preparation

	Per cent Phenol
Dialyzed serum after addition of 0.3 per cent phenol	0.346
Supernatant of acid-protein precipitate	0.320
Acid-protein precipitate moist	0.432

Experiment 3. Antipneumococcus (Type VIII) Preparation

	Per cent Phenol
Original serum	0.298
Serum after dialysis—containing precipitate	0.098
Supernatant of dialyzed serum	0.088
Moist precipitate of dialyzed serum	0.222
Mixture containing antibacterial precipitate pH 5.9	0.233
Moist antibacterial precipitate	0.234

Experiment 4. Antipneumococcus (Type V) Preparation

	Per cent Phenol
Serum after dialysis and acid protein precipitation	0.368
Antibacterial substance precipitated at pH 6.1 by addition of 4 volumes of distilled water containing 0.2 per cent phenol	
Supernatant of antibacterial precipitate	0.232
Moist antibacterial precipitate	0.342

Some results on the phenol contents of preparations before and after dialysis (in cellophane bags against running water for six days) are presented in Table I. In every case phenol was present after dialysis in concentrations ranging from 0.032 to 0.118 per cent.

TABLE I

EXPERIMENT NO.	MATERIAL	VOLUME ML.		PER CENT PHENOL	
		INITIAL	FINAL	INITIAL	FINAL
1	Antipneumococcus serum	1,040	1,140	0.262	0.094
2	Antipneumococcus serum	18,600	22,000	0.246	0.032
3	Antipneumococcus serum	19,500	23,000	0.298	0.098
4	Antipneumococcus serum	20,000	23,000	0.296	0.094
5	Antistreptococcus serum	720	770	0.482	0.118
6	Refined antipneumococcus preparation			0.504	0.070
7	Pneumococcus antibacterial substance containing 0.5 per cent phenol, precipitated by 50 per cent saturation with ammonium sulfate solution		1,250		0.048

DISCUSSION

It may be stated that the estimation of the phenol in the mixtures is accurate to 2 per cent of the amount present or better. The calculation of the concentrations, however, should be defined more clearly than is the case generally. In the analytical determinations presented here, per cent phenol was given as grams per 100 c.c. of solution, mixture, or precipitate. On the other hand, when added to serum in the routine procedures, the addition of phenol is calculated as per cent by volume; that is, nine parts of melted phenol are diluted with one part of water, and this solution is considered 90 per cent phenol by volume. If it is desired, for example, to have 1,000 c.c. of serum containing 0.2 per cent phenol by volume, it would be necessary to add 2.22 ml. of the 90 per cent phenol solution, which would correspond to 2.0 ml. phenol or 2.13 gm.

The results show clearly that phenol is carried down, or adsorbed, by precipitating protein to a concentration at least equal to, and generally considerably greater than, that present in the solution.

It will hardly be necessary to discuss the experimental findings in detail. Antipneumococcus preparations were mainly used in this study because they were available in considerable numbers at the time. There is every reason to believe that other antibody preparations, protein materials of different kinds, and even nonprotein material, such as aluminum hydroxide, when precipitated from solutions containing phenol, would carry down appreciable quantities of phenol which must be reckoned with in the later manipulations.

The data for Experiment 1 are given in fairly complete form. It is evident that the precipitate from dialysis, the acid-protein precipitate, and the antibacterial-protein precipitate, carry down phenol in concentrations greater than would be expected from its concentration in the solutions. Calculations are given to show the amounts of phenol present in the final preparations if the usual method, as indicated earlier in this paper, of adding additional phenol is followed. Experiments 2, 3, and 4, show similar results in more condensed form.

In the dialysis of the various protein preparations, it is generally assumed that phenol is removed in the course of the dialysis. The data from Experiments 1 to 7 show that this is not entirely correct. Even after six days of dialysis, considerable concentrations of phenol may be retained; between 0.032 and 0.118 per cent in the results given. This is of importance in connection with the precipitation of antitoxin and antibacterial proteins by ammonium sulfate (or other precipitant) where, if phenol is present, it would in all probability be adsorbed by the precipitating protein. The phenol would not be removed completely in the subsequent dialysis, and should be considered when adding preservative to the desired concentration.

In view of the experiments described in this paper, it may be stated that the phenol content of protein (and other) materials containing phenol as preservative should be controlled during their refining and concentration. This is especially important for the antitoxin and antibacterial preparations intended for therapeutic use.

SUMMARY

The phenol contents of a number of antibacterial preparations at various stages of refining and concentration were determined. It was found that considerable concentrations of phenol were carried down, or adsorbed, by the separated proteins. Special care must, therefore, be exercised in the addition of phenol to the final therapeutic products.

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EFFECT OF HEAT ON THE HEMOLYTIC AND SKIN-NECROTIZING FACTORS IN STAPHYLOCOCCUS TOXIN*

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MANY observations have been made on the effect of heat on staphylococcus toxin; however, these usually have been limited to determining whether or not a fraction of the toxin has been destroyed at a specific temperature after a definite interval. Systematic studies on the effect of heat on many of the hemolysins have been made by Madsen and his co-workers. Included in their group are vibrolysin, tetanolysin, streptolysin, and epeiralysin.[1]

The present investigation was undertaken following the suggestion of Dr. Thorvald Madsen. The methods used are essentially the same as those used by him in observing the effect of heat on hemolysins.[2] This study shows the effect of heat on the hemolytic and skin-necrotizing factors in staphylococcus toxin.

METHODS AND MATERIAL

The staphylococcus toxin was supplied through the courtesy of Lederle Laboratories and was prepared as follows: Cultures of *Staphylococcus aureus* were inoculated on a semisolid agar under a mixture of 20 per cent carbon dioxide in oxygen for forty-eight hours at 37.5° C. The medium contained 1000 c.c. veal infusion, 2.0 per cent Difco proteose peptone, 0.5 per cent sodium chloride, 0.7 per cent sodium acetate, and 0.2 per cent agar. The pH was adjusted to 7.4.

After incubation, the agar was separated from the liquid by filtration in the cold, and the filtrate was passed through a Mandler filter to render it bacteria free.

The toxin was preserved with merthiolate in a final dilution of 1:10,000, and at the time of preparation had the following potency: hemolytic titer 1:900, dermonecrotic titer 1:800, and acute killing dose (intravenous) 0.05 c.c.

The water bath was thermoelectrically controlled. The temperature never varied more than 0.02 to 0.08° C. during any experiment. The temperature at which the toxin was heated is given in the experiment.

Approximately 40 c.c. of the staphylococcus toxin was put into a pyrex glass cylinder. It was then placed in the water bath. A Beckmann thermometer and a pyrex glass stirring rod were placed in the toxin through two holes in the stopper. An electric motor was used to stir the toxin at a constant speed. When the temperature of the toxin in the cylinder reached the temperature of the water bath, 2 c.c. were removed. At definite intervals, usually ten minutes, subsequent samples of toxin were taken. Some of the experiments ran as long as one hundred and fifty minutes. The samples of toxin were placed immediately in an ice bath (-0° C.). The toxin froze after a short time. The tubes

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were then placed in the icebox (5° C.), and usually kept for twenty-four to seventy-two hours before the hemotoxin titrations were made.

Rabbit's red cells were used for the indicator in the hemolytic test. The cells were washed three times in physiologic saline and suspended in a similar salt solution. One cubic centimeter of a 2 per cent suspension of these cells was added to each tube.

TABLE I

AMOUNT OF STAPHYLOCOCCUS TOXIN NECESSARY TO PRODUCE THE SAME DEGREE OF LYSIS AT ANY OF THE FOLLOWING TEMPERATURES AND THE RESIDUAL HEMOTOXIN EXPRESSED IN PER CENT

TIME MINUTES	45° C.		47.4° C.		52° C.		53° C.		54.03° C.	
	TOXIN IN C.C.	% RESIDUAL TOXICITY	TOXIN IN C.C.	% RESIDUAL TOXICITY	TOXIN IN C.C.	% RESIDUAL TOXICITY	TOXIN IN C.C.	% RESIDUAL TOXICITY	TOXIN IN C.C.	% RESIDUAL TOXICITY
0	0.000126	100.0	0.000126	100.0	0.000126	100.0	0.000126	100.0	0.000266	47.3
10	0.000128	98.4	0.000126	100.0	0.000143	88.1	0.000326	38.6	0.000623	20.2
20	0.000138	91.3			0.000164	76.8	0.00044	28.6	0.001216	10.3
30	0.000128	98.4	0.000160	78.7	0.000234	53.8	0.000743	16.9	0.002044	5.6
40	0.000128	98.4			0.000244	51.6	0.000883	14.2	0.003833	3.2
50	0.000136	92.6	0.000153	82.3			0.001193	10.5	0.006266	2.0
60	0.000132	95.4			0.000365	34.5	0.001700	7.4	0.008233	1.5
70	0.000153	82.3	0.000210	60.0	0.000568	22.0	0.002466	5.1	0.009330	1.3
80	0.000155	81.2			0.000577	21.8	0.002866	4.3	0.013330	0.94
90	0.000138	91.3	0.000206	61.6	0.000676	18.6	0.003600	3.5	0.015660	0.80
100	0.000145	86.9			0.000825	15.3	0.004100	3.07	0.019660	0.64
110	0.000136	92.6	0.000226	54.8	0.000833	13.8	0.005100	2.4	0.024000	0.52
120	0.000138	91.3			0.000900	14.0	0.008660	1.4	0.025660	0.41
130			0.000195	64.6						
140										
150	0.000135	93.3	0.000230	54.7						
	54.05° C.		55° C.		55.65° C.		56.35° C.		57° C.	
0	0.000300	42.0	0.000633	19.8	0.001500	8.4			0.001266	9.9
10	0.000766	16.4	0.001600	7.8	0.003930	3.2	0.004500	2.8	0.006160	2.0
20	0.001580	7.9	0.004110	3.06	0.007560	1.6	0.009000	1.4	0.014500	0.87
30	0.002433	5.1	0.008260	1.5	0.011600	1.0	0.012000	1.0	0.027000	0.46
40			0.011430	1.1	0.013300	0.94	0.017500	0.77	0.034330	0.36
50			0.015000	0.84	0.017300	0.78	0.024000	0.52	0.049000	0.25
60			0.017160	0.73	0.019839	0.68	0.027000	0.46	0.060000	0.21
70	0.008060	1.5	0.026580	0.47	0.022160	0.56	0.031000	0.40	0.060000	0.21
80	0.013000	0.97	0.027160	0.46	0.028660	0.44	0.040000	0.31	0.070000	0.18
90	0.018580	0.67	0.035800	0.35	0.026160	0.48	0.042500	0.29	0.067500	0.17
100	0.021260	0.58	0.046600	0.27	0.033660	0.37	0.046500	0.27	0.090660	0.13
110	0.023160	0.54	0.044000	0.28	0.032330	0.38	0.056500	0.22	0.103300	0.11
120	0.026000	0.48	0.046330	0.27	0.042200	0.29	0.063000	0.2	0.105000	0.11
130							0.075000	0.168		
140							0.080000	0.157		
150										

The toxin was titrated over a range varying from 0.8 c.c. to 0.0001 c.c. It was diluted with physiologic saline. The volume in each tube was kept constant by the addition of a 0.85 per cent solution of sodium chloride. The test tubes were uniform in size and measured 1 by 10 cm. The red cells were added to the dilution of toxin, and the tubes were kept at room temperature (27° to 32° C.) overnight before the degree of lysis was recorded.

The amount of lysis which occurred in this period was determined by quantitative methods. Usually three tubes which showed only a small amount of

hemoglobin were selected for the standards from the series heated for the shortest period at each of the different temperatures. The amount of hemoglobin in each tube in all the series of titrations heated at the same temperature was compared with these standards. The average of the amount of staphylococcus

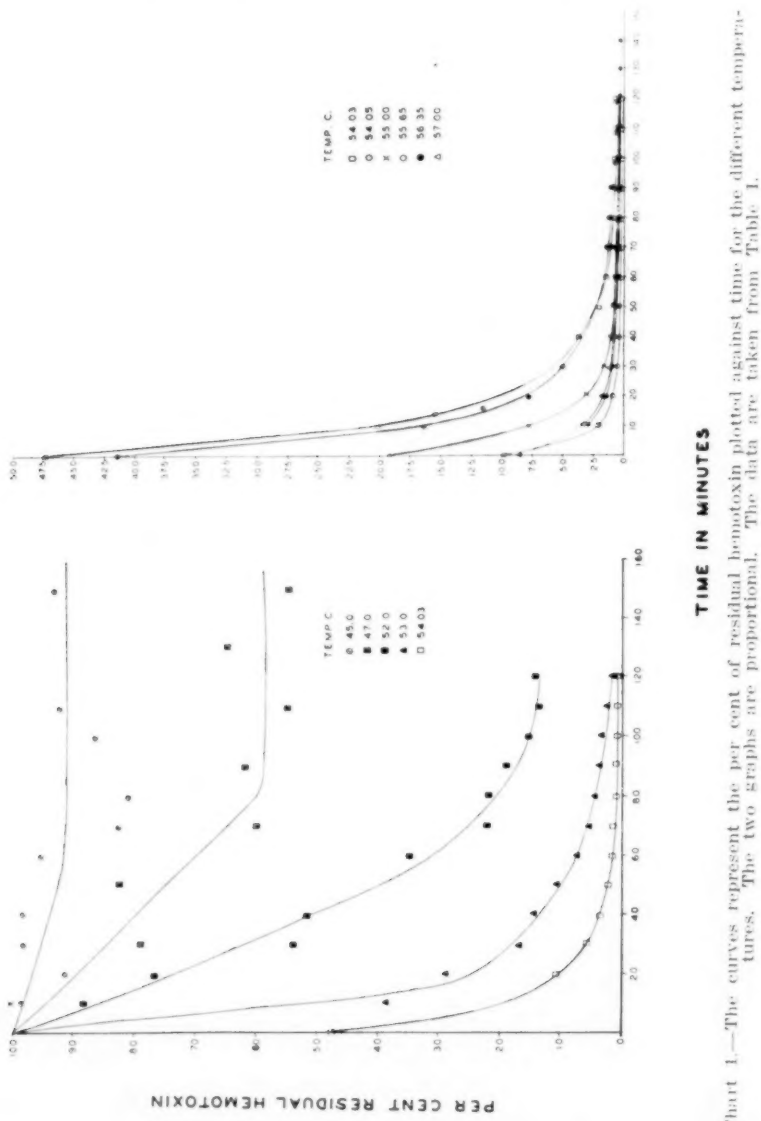

Chart 1.—The curves represent the per cent of residual hemotoxin plotted against time for the different temperatures. The two graphs are proportional. The data are taken from Table 1.

toxin in the three tubes was taken in each series which matched the standards. This quantity of toxin was considered to be necessary to produce the same degree of lysis as that in the standard tubes for that particular temperature.

The percentage of residual hemotoxin in any sample was determined by dividing the volume of toxin in the specific tube into the amount of toxin

necessary to produce the degree of lysis which was considered to represent 100 per cent toxicity of the unheated toxin.

The amount of hemoglobin was determined by comparing directly the color in the different tubes.

The effect of heat on the skin-necrotizing factor in staphylococcus toxin was determined by injecting intradermally into two rabbits 0.1 c.c. of the different samples of the toxin in which the amount of residual hemotoxin was known.

TEMP. C.	TIME IN MINUTES	0	10	20	30	40	50	60	70	80	90	100	110	120
53°	% RESIDUAL HEMOTOXIN						10.5	7.4	5.1	4.3	3.5	3.07	2.4	1.4
	SKIN NECROSIS													X
54.03°	% RESIDUAL HEMOTOXIN			10.3	5.6	3.2	2.0	1.5	1.3	0.94	0.80			
	SKIN NECROSIS							X	X	X	X			
55°	% RESIDUAL HEMOTOXIN	19.8	7.8	3.06	1.5	1.1	0.84	0.73	0.47					
	SKIN NECROSIS						X	X	X					
57°	% RESIDUAL HEMOTOXIN	9.9	2.0	0.87	0.46	0.36	0.25	0.21	0.21					
	SKIN NECROSIS			X	X	X	X	X	X					

Chart 2.—Skin necrosis in the rabbit following the intradermal injection of staphylococcus toxin heated at the different temperatures for varying intervals of time compared with the residual hemotoxin. No necrosis occurred in the areas indicated by X.

EXPERIMENTAL

Table 1 shows the amount of staphylococcus toxin heated at temperatures between 45° and 57° C. that is necessary to produce the same degree of lysis for any specific temperature. It also gives the amount of residual hemotoxin expressed in per cent.

Chart 1 shows the results obtained when the residual hemotoxin is plotted against time in minutes.

The areas of skin necrosis produced by the different samples of toxin are shown in Chart 2. It can be seen from these data that the size of the necrotic areas are proportional to the amount of the residual hemotoxin. Only samples of toxin heated for different intervals at 53°, 54.03°, 55°, and 57° were used since only a limited number could be injected intradermally into a single animal.

DISCUSSION

The physicochemical views with regard to toxin and antitoxin advanced by Ehrlich,[3] Arrhenius and Madsen,[4] and Bordet,[5] about the beginning of the twentieth century, have brought forth considerable discussion. Our knowl-

edge of such processes even at present is very limited. Little has been contributed since 1900 as to the composition of toxins. Until more information is obtained with regard to these substances called toxins, it appears of little value to attempt to determine the type of reaction obtained when staphylococcus toxin is heated.

The data given in Table I show that staphylococcus toxin is thermolabile. Chart 1 shows that the rate of detoxification is influenced by the temperature and the length of time it is heated. The hemolysin is destroyed very rapidly during the first thirty minutes when the temperature is 53° C. or higher. The residual hemolysin at this time apparently is more resistant to heat. Only a slight decrease occurs in the amount of hemotoxin when this same toxin is heated at 45° for one hundred and fifty minutes.

The rate of detoxification of staphylococcus toxin by heat suggests that there may be two hemolytic agents present which vary in their thermolability.

Schrek,[6] in studying heat inactivation of tetanolysin, found that the first 99 per cent was inactivated at a constant rate and the last 1 per cent was inactivated slower. He suggested that the accumulation of the products of heat inactivation decreased the rate of the reaction.

It is of interest to note that the skin-necrotizing factor in staphylococcus toxin is destroyed by heat. The rate at which it is detoxified runs essentially parallel with the rate at which the hemolysin is destroyed. The results of the effect of heat on the hemolysin and skin-necrotizing factors in staphylococcus toxin suggest that they may be the same substance. Apparently no one has been able to determine conclusively that they are the same or different substances.

SUMMARY

The hemolysin and the skin-necrotizing factor in staphylococcus toxin are both detoxified by heat. The rate at which these fractions are destroyed is essentially parallel. It is suggested that they may be the same substance.

The rate at which the hemotoxin is destroyed is influenced by the temperature and the length of time the toxin is heated.

The rate of detoxification of staphylococcus toxin by heat suggests that there may be two hemolytic substances which vary in their thermolability.

I wish to express my appreciation to Dr. Arthur C. Omberg, Physicist in the Roentgenological Department of Vanderbilt University, for his assistance in this study, and to the Department of Physiology and Pharmacology which supplied the equipment for this experiment.

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SIMULTANEOUS BILATERAL SPONTANEOUS PNEUMOTHORAX

Report of a Recurrent Case Due to Congenital Cystic Lung Disease

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IN 1934 Oechsli and Miles reported a case of recurrent simultaneous bilateral spontaneous pneumothorax due to congenital cysts of the lung. At the time of this report they found reported in the literature a total of 77 cases of bilateral spontaneous pneumothorax, two of which were due to cystic lung disease. Glickman and Schlomovitz, in 1936, also reviewed the literature on simultaneous bilateral pneumothorax and reported a total of 82 cases, three of which were due to congenital cystic disease of the lungs. We wish to add another case of this rare entity, the diagnosis of which was made before death and confirmed at autopsy.

A white male student, aged 17 years, was first seen in our office September 25, 1933, complaining of shortness of breath and fatigue. The family history and past history were not significant. He had been in good health until the early summer of 1933, when he first noticed that he tired easily. This fatigue became progressively more noticeable, and he soon developed severe shortness of breath for which he had been hospitalized. X-ray examination at that time revealed a bilateral pneumothorax, with complete collapse of the right lung and the upper one-third of the left lung. Symptomatic relief had promptly followed the removal of several hundred cubic centimeters of air from the right pleural cavity.

When seen by us physical examination revealed a rather thin white male, weighing 114 pounds. He was dyspneic on slight exertion, but there was no evidence of cyanosis. He had only a degree of fever, and his pulse rate was only 96. His heart appeared entirely normal. We call attention to this because of later developments. The lung findings were those of a pneumothorax over the upper two-thirds of the right lung. Over the left lung there was some increase in the voice and breath sounds and a few scattered small moist râles. Otherwise the physical examination was negative. Laboratory studies of the blood and urine were normal.

Fluoroscopic observation and roentgenograms of the chest showed a rather uniform collapse of the upper portion of the right lung by pneumothorax, about 60 per cent by volume. There was scattered fibrosis in the uncollapsed portion of this lung, and a generalized fibrosis throughout the left lung, with numerous small areas of lessened density, giving the appearance of honeycombing. The absence of calcium was conspicuous. The patient had a right-sided pneumothorax which, from the history, was spontaneous and also bilateral. The lesion in the lungs was so unusual that cystic lung disease was considered the probable diagnosis.

In order to give the patient further study he was temporarily kept at bed rest and gradually returned to a relatively normal routine. Because of irregularity of expansion and a tendency to localized ballooning of the lung tissues, small pneumothorax refills were given and the lung permitted to re-expand gradually over a period of several months. During this time the patient was not permitted to return to school but otherwise participated in his usual

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activities. Study of lungs following complete re-expansion showed an unusual type of fibrosis with honeycombing which we definitely diagnosed as congenital cystic lung disease.

The patient's course continued uneventful for about a year when he noticed a return of symptoms. He reported to the office and was found to have a bilateral spontaneous pneumothorax, with about 90 per cent collapse of the right lung and 30 per cent collapse of the left. Withdrawal of 600 c.c. of air from the right pleural cavity relieved symptoms of respiratory embarrassment, so patient was not hospitalized. The following night he became suddenly worse. When seen at his home soon thereafter, he was found sitting in a chair gasping for his breath. One thousand cubic centimeters of air were removed from the right pleural cavity, which relieved his acute distress; the patient was immediately taken to a hospital. On arrival at the hospital he was cyanotic and again gasping for air. Both pleural cavities were aspirated and a needle retained in the right pleural cavity and connected to a water bottle to permit constant aspiration on this side. Despite this, and repeated aspirations on the left side, dyspnea and cyanosis again returned, and it was necessary to connect the left pleural cavity with a water bottle for continuous aspiration. By continuing constant aspira-

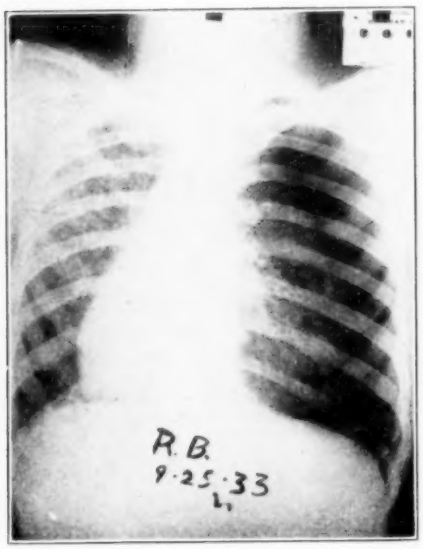

Fig. 1.

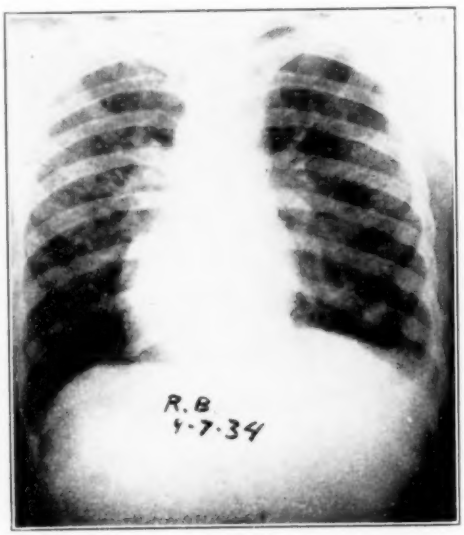

Fig. 2.

Fig. 1.—R. B. 9-25-33. Film made when first seen shows pneumothorax on right; generalized fibrosis with multiple honeycombing throughout both lungs.

Fig. 2.—R. B. 4-7-34. Right lung almost completely re-expanded.

tion of both pleural cavities the patient soon became comfortable and made an uneventful recovery. He was discharged from the hospital five weeks after admission, although neither lung had completely re-expanded.

In January, 1935, x-ray showed the right lung had completely re-expanded, but there was a small area of pneumothorax over the left upper lobe. A lung mapping with lipiodol at this time showed the oil filling numerous small dilated areas in the lower portion of both lungs. This finding, coupled with failure to find tubercle bacilli in the sputum after repeated search over a period of one and a half years, confirmed our impression that we were not confronted with tuberculosis; since the patient was now symptom free, he was permitted to return to school.

Patient's course continued uneventful for the next nine months when he developed an acute respiratory infection manifested by cough, expectoration, dyspnea, and fever. Lung findings at this time were those of an acute tracheobronchitis. Examination of the heart revealed an apical systolic murmur and a definite accentuation of the second pulmonic sound.

Electrocardiogram was normal. Although he recovered from this infection, he had recurrences, and his dyspnea became progressively worse. Five months later x-ray examination revealed that both lungs had completely re-expanded and for the first time showed evidence of definite cardiac enlargement.

A few months later, while visiting in another city, the patient had another similar acute respiratory infection and was seen by physicians who insisted that he had tuberculosis. Because of this difference of opinion concerning the diagnosis, the patient was admitted to a tuberculosis sanatorium in September, 1936. While under treatment there, he developed signs of progressive cardiac decompensation from which he died in May, 1937.

Autopsy was limited to contents of thorax. There was a moderate amount of edema of the lower extremities and genitalia. On opening the thorax the visceral and parietal pleurae were densely adherent, so that the parietal pleura had to be removed with the lungs. The diaphragm and pericardium were also adherent to the lungs. On opening the pericardium there was found about 100 c.c. of clear straw-colored fluid.

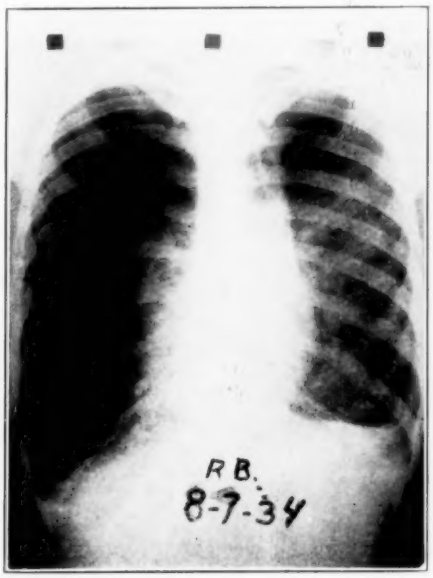

Fig. 3.

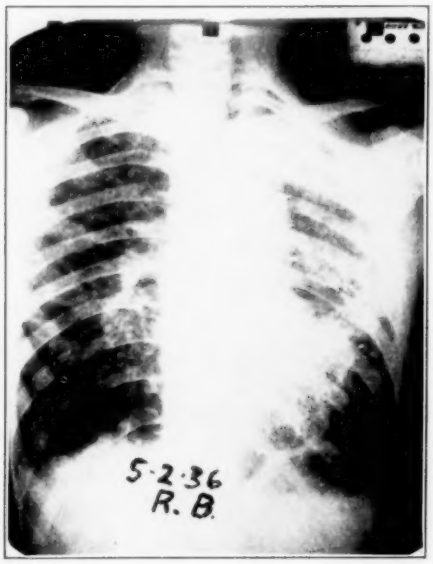

Fig. 4.

Fig. 3.—R. B. 8-7-34. Simultaneous bilateral spontaneous pneumothorax. Large pneumothorax on left and smaller one on right.

Fig. 4.—R. B. 5-2-36. Film made in oblique position. Both lungs completely re-expanded. Cystic areas in midzone in each lung.

Heart: The heart appeared to be moderately enlarged, particularly on the right side. It weighed 440 gm. after opening. The epicardium was smooth and glistening, and there was a small amount of fat present in the coronary sulci. The myocardium was dark red in color and showed no evidence of scarring. Thickness of right ventricle was 5 mm. and thickness of left ventricle was 1 cm. The right auricle and ventricle were tremendously dilated. The left auricle and ventricle appeared to be normal, except for a slight dilatation of the left ventricle. The endocardium was thin, smooth, and glistening. The papillary muscles of the right ventricle were greatly thickened. The columnae carneae were also thickened. Those of the left ventricle appeared normal. The chordae tendineae were neither shortened nor thickened. The valves were thin and veillike and showed no evidence of any disease. The valves appeared competent. The circumference of the valves measured as follows: tricuspid 12.5, pulmonic 7.5, mitral 9, and aortic 6 cm. There appeared to be some slight dilatation of the pulmonary cornice. There were no congenital or other abnormalities of the great vessels.

Aorta: The ascending aorta, the arch, and the thoracic portion of the descending aorta appeared normal. They were thin and elastic, and showed no evidence of arteriosclerosis. The coronary vessels appeared normal.

Lungs: The visceral and parietal pleurae were densely adherent and appeared greatly scarred. Large cystic areas in the lungs could be seen and felt beneath the pleura. They apparently contained air and no fluid. Both lungs appeared to be overdistended and maintained their shape. The lobes of the lungs also showed dense interlobar adhesions. The lungs were filled with formalin and allowed to fix before further sectioning. After sectioning, the cut surface of the lungs showed both lungs to be greatly involved by a cystic change. These cysts were most numerous and largest at the periphery of the lungs. They varied greatly in size from that of normal vesicles up to cysts 3 cm. in diameter. The walls of these cysts were thin and smooth. They apparently communicated with the bronchi. The normal lung tissue was brownish red in color, presumably due to fixation with formalin. There was no evidence of any infection anywhere in either lung or in the tracheobronchial lymph nodes.

To open the thorax, it was necessary to extend the incision down to the abdomen, and this opportunity for exploring the abdomen was taken. The peritoneal cavity was filled with a clear amber-colored fluid. The liver seemed to be enlarged, but the other organs appeared to be normal as far as could be determined by palpation.

Microscopic.—Heart: Left ventricle, including coronary artery and portion of mitral valve, was normal. Right ventricle, including tricuspid valve, showed only very slight scarring of myocardium. *Aorta:* Sections from ascending portion and thoracic descending portion were normal. *Pulmonary Artery:* Moderate proliferation, scarring, and slight round cell infiltration of intima. Media appeared somewhat distorted and edematous. *Lungs:* The alveoli varied in size from normal to large cystic structures. They were lined by normal to greatly thinned out and flattened cells. The transition from small bronchioles to alveoli could be seen. The small bronchioles were lined by very small ciliated columnar epithelial cells, much smaller in size than the cells lining the larger bronchi. These cells became flattened and lost their cilia at the point of transition from bronchiole to alveolus. These small bronchioles were greatly dilated. No definite constrictions could be found. The walls of the alveoli were very thin and delicate. There were many heart failure cells in some of the alveoli. The interlobular connective tissue was edematous and much greater in amount than normal. It was slightly to moderately infiltrated by lymphocytes, with occasional focal accumulations of these cells. The blood vessels and lymphatics were numerous and dilated. Some showed hemorrhages. The larger branches of the pulmonary arteries showed the same changes as in the main branch. The pleura was slightly to greatly thickened and vascularized. It was slightly infiltrated by lymphocytes. *Tracheobronchial Lymph Nodes:* Contained a small amount of anthracotic pigment. There were many large mononuclears containing blood pigment in the sinuses. They were otherwise normal.

Anatomic Diagnosis: Congenital cystic disease of the lungs; chronic fibrous pleurisy; hypertrophy and dilatation of the right ventricle; chronic passive congestion of lungs; pericardial effusion; edema of lower extremities and genitalia, ascites.

COMMENT

Simultaneous bilateral spontaneous pneumothorax from any cause is an unusual accident and, when resulting from congenital cystic lung disease, it is relatively rare. The case here reported presents several interesting features, many of which are similar to that of the case reported by Oechsli and Miles. The patient remained in good health until he was 17 years of age, when the first spontaneous pneumothorax occurred. He lived for four years after this, and died of congestive heart failure. Following the first occurrence of spontaneous pneumothorax, the lungs completely re-expanded before the second bilateral spontaneous pneumothorax occurred. Following the second occurrence of this accident, the lungs again completely re-expanded, and remained so for several months before death. So far as we have been able to find, this is

the second case on record in which this has happened. There was a striking absence of cough, expectoration, fever, and chest pain during the greater part of the time he was under observation, and when these symptoms did occur, they were easily accounted for by an acute respiratory infection. Unlike the case of these authors, there were no other congenital abnormalities present.

The successful treatment of spontaneous pneumothorax may require removal of air from the pleural cavity, either by repeated or continuous aspiration. Simple pneumothorax, with a small tear in the lung, may need no treatment, or at most one or a few aspirations of air. When the lung tear has closed, the air will be absorbed from the pleural cavity. Larger tears with tension pneumothorax require continuous aspiration with a needle, or rarely with a catheter, inserted into the pleural cavity and connected with a water bottle, such as is used in the drainage of an empyema. The end of the tube should be placed just below the level of the water to permit continuous escape of the air, even though the intrapleural pressure should approach normal. With reasonable aseptic precautions, infection of the pleural cavity can be avoided. Although this patient repeatedly had needles inserted through the chest wall and for several days needles were left in place in both pleural cavities, no infection occurred, confirming our impression that the danger of infection from without is negligible.

The diagnosis of congenital cystic lung disease presents little difficulty when large cysts are present. In the type of the disease characterized by multiple small cysts the diagnosis may be confusing as the condition may simulate an infectious process, especially tuberculosis. In this case a positive tuberculin reaction further confused the picture. No evidence of tuberculosis, however, was found at autopsy.

There is no satisfactory treatment for this type of cystic lung disease, and the prognosis is poor. The treatment of this patient was symptomatic and the necessity for maintaining constant aspiration from each pleural cavity indicated that there was no communication between the pleural cavities through the mediastinum.

SUMMARY

A case of simultaneous bilateral spontaneous pneumothorax resulting from congenital cystic lung disease is reported with autopsy findings. The patient had a bilateral collapse on two different occasions, and following each the lungs completely re-expanded. The lungs had been completely re-expanded for several months before death occurred. Death was directly the result of congestive heart failure. At autopsy no other congenital abnormalities were encountered.

We wish to express our indebtedness to Dr. C. L. Harrell of Norfolk, Va., who treated the patient for his first bilateral pneumothorax, for the notes on this patient; to Drs. W. E. Brown and F. B. Stafford of Blue Ridge Sanatorium, under whose care the patient was at the time of death; and to Dr. W. N. Weaver of University, Va., who performed the autopsy.

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CORRELATION OF HISTOLOGIC STRUCTURE WITH CLINICAL FEATURES*

II. The Case of Malignant Neoplasms

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THE structure of malignant new growths, which is revealed by the classical histologic methods of study, forms the basis of the prevailing conceptions as to their nature. Modifications of these conceptions become necessary whenever some essential improvement in technique appears. Tissue cultures have revealed unexpected properties in the tumor cells. The wet-film method applied to absolutely fresh tissue has shown that many of the cells in the neoplasm are not rigidly fixed within a particular kind of architecture, and has disclosed objects previously not suspected. Perhaps of all the pictures, that which dark-field illumination provides is the most striking, because the intensely active movement of the particles in the neoplasm is thus for the first time brought to light.

Histologic structure cannot be altogether divorced from physiologic behavior. The phenomena observed during life must go with corresponding cellular elements, so that observation can only have been imperfect if these events do not accord with the clinical phenomena. This view was discussed in connection with goiter‡ where it is very evident that the clinical pathologist's findings are widely at variance with the clinician's observation as to presence or absence of toxic symptoms, etc. It was shown that a more exact analysis of thyroid tissue would bridge the discrepancies. Starting with the thyroid unit, and filling in the variable details, all gradations of disease can be amply provided for.

So, too, with cancer. Only, in the case of malignant neoplasms, we distinguish two series of components, one corresponding to the parenchyma of organs, and the other consisting of superadded elements not ordinarily found in noninfective diseases. There is an essential dualism in the structure.

Discarding for the moment the stereotyped classification of tumors, the first step in the analysis is to catalogue the various elements which ever occur in tumor tissue. The next step is to assess the relative degrees of importance of each element and determine its chronologic relation to every other. From these data the essential features of each case can be put together into a simple formula—following the method proposed in the paper on goiter. Finally, the various formulas are compared, analyzed, and correlated with the clinical

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data, with the ultimate object of seeking the explanation of the various features of the disease in the given patient and the nature of malignancy in general.

HISTOLOGIC CANCER UNIT

The histologic unit of cancer tissue comprises cells and a matrix carrying vascular channels and nerve filaments. The cellular components may be grouped into (1) fixed, (2) free-floating tumor cells, and (3) wandering cells (histiocytes).

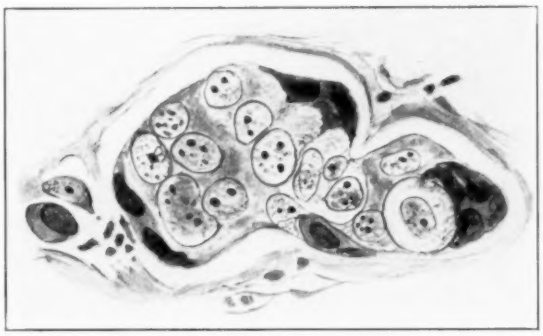


Fig. 1.—Carcinoma solidum of breast. Growing point of one of the cell columns. Slightly schematic drawing to show the dusky trophic cells and the paler-staining "essential" cells. This depicts the "cancer unit" (oil immersion, Oc. 15).

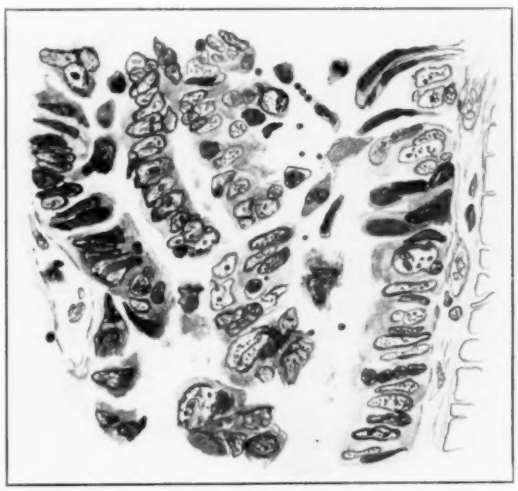


Fig. 2.—Adenocarcinoma of colon. Slightly schematic drawing of a portion of an acinus (cancer unit) to show the dusky trophic cells and paler-staining "essential" cells. Many free cells are shown, and some chromidial granules (oil immersion, Oc. 15).

1. *Fixed cells* vary in shape in the different classes of neoplasm (squamous, spheroidal, and columnar-celled, etc.), and in each case are of two kinds: (a) the trophic cells or trophocytes, which have a dusky cytoplasm, and a relatively large pachychromatic nucleus; (b) the essential or proper cells, which are larger, show a pale-staining cytoplasm, and a relatively smaller leptochromatic nucleus. (Figs. 1 and 2.) This division is general in both animal and vegetal organs. Thus, in normal histology, the Sertoli cells of the testis, the basal cells of the epithelium, the argentaffine cells of the neuro-

endocrine systems are trophic cells, and the germ cells, epithelial cells, and ganglionic cells are the respectively corresponding essential cells. During youth, food material normally flows from trophocyte to essential cell; in senescence the flow is in the opposite direction, according to Borrel. In addition to these fixed cells, many malignant neoplasms also show giant cells with bizarre nuclei; these may, however, also be of the nature of trophocytes.

2. *Free-Floating Cells.*—These comprise (a) small and large "naked" nuclei; (b) small triangular cells like miniature plasma cells; (c) foamy cells,

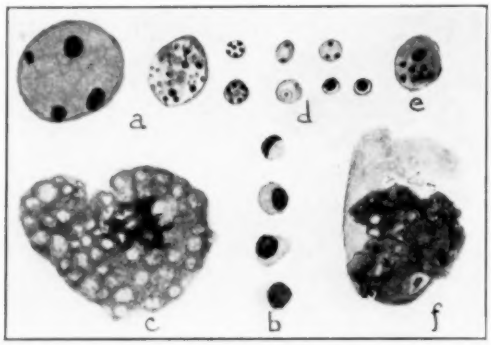


Fig. 3.—Free-swimming cells: *a*, *a'*, large and small "naked" nuclei; *B*, cells like plasma cells; *c*, foamy cell. Chromidia: *d*, type "Ib" and "Ic," *E*, type "Ie," "octet." Filamentous forms: *f*, noded lines in cancer cell nucleus and cytoplasm (Zeiss, oil immersion, Oc. 15).

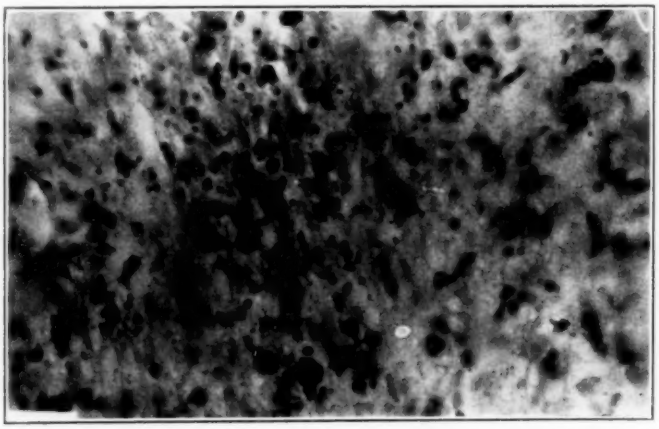


Fig. 4.—Sarcoma. (a) To show the granulations of types Ib and Ic (small and large granules). The latter have a narrow halo. (Zeiss lens, obj. 20, Oc. 15.) (b) Another preparation showing larger granulations among the tumor cells.

some full of fat droplets and amoeba-like in form, others containing other granules and no fat; (d) tailed cells (Fig. 3).

These cells can only be identified in wet film or in vital stained preparations. In other words, the operation material must be subjected to cytologic analysis within five minutes of removal from the body, if all these cell forms are to be demonstrated. Smear preparations made like blood smears (with or without dilution in isotonic saline) show them quite well, and dark-field study is advisable on other portions of material as soon as the films, etc., have

been safely deposited in the fixing fluids. (The wet-film method[1] consists in plunging the still wet tissue scrapings into Schaudinn, or Bouin, for subsequent staining by Mayer's hemalum or by iron-hematoxylin—the most informative nuclear stain.)

(a) *Naked nuclei.* These vary much in size. Their outline is circular or oval or indented on both sides. Some have coarse granules of chromatin, with clear intervening material, and resemble the embryonic forms of some protozoan cells of stagnant water. Others show flaky or tigroid markings, with a color reaction to Leishman, suggestive of their being dying cells, whose cytoplasm has been reduced from a mere halo to the vanishing point. The small germ center cells of lymph nodes (lymphoidocytes) often present the same appearance in film preparations of lymphoid tissue. Nucleolar matter is present. Some of these cells may appear as round shells enclosing a bundle of pyriform bodies. In the dark field, some of these cells have a gregarine-like appearance, and alter their shape continually, indicative of irritability.

(b) *Miniature plasma cell-like forms.* These are triangular, the nucleus being round, trachychromatic and pachychromatic, and forming the bulk of the cells, while the cytoplasm is dusky. They are seen best of all by mingling the fresh scraping with the methyl green pyronine stain of Pappenheim, and immediately covering the mixture with a cover slip.[2] After a short time the different cells become clearly distinguishable.

(c) *Foamy cells.* The most striking are found in breast tumors and show protoplasmic masses full of relatively large fat globules (which also occur free throughout such films), and show one or two quite small nuclei. Others appear foamy in virtue of being packed with refractile granules, but differ in failing to give a fat reaction.

(d) *Tailed cells.* These were known to the earliest histologists. (They figure in L. S. Beale's plates published in 1870.) Some of them are of very large size and contain inclusion bodies—either scattered (Fig. 7) or in aggregated masses like included protozoa. The latter would be described as the normal segregation apparatus of the cell by some cytologists.

In passing, it may be noted that these various free-floating cells are all referred to in J. Koch's monograph[3] as successive stages in the development of a protozoan parasite, the cellula cancrosa; in other words, he regards them as foreign cells and not host cells at all. Other observers in recent years have come to realize that organisms can pass from ultramicroscopic dimensions through all gradations up to the size of the largest body cells.

3. *Wandering cells* include macrophages, monocytes, histiocytes, and various inflammatory cells. The monocytes are a very striking feature of cancer tissue cultures, moving as they do among and all over the tumor cells. In some cases, in fact, the tumor appears actually to be composed of macrophages. In histologic sections, however, such cells may escape notice or are only recognized by their nuclear form—the cytoplasm being relatively inconspicuous. The nuclei are liable to be mistaken for the granulations presently to be discussed, and when the macrophages are active, the engulfed objects may be

mistaken for specific inclusions. The number and varieties of the inflammatory cells in general depend on the concurrent secondary infection in the neoplasm, but the cell formula is worthy of record as part of the histologic analysis of a given laboratory specimen.

These macrophages are, of course, not specific for cancer, but the fact that they are much more plentiful in malignant neoplasms than in septic, tuberculous, and other tissues gives them a quasi-specificity.

CHROMIDIAL GRANULATIONS

To this point the cancer unit has been described as to its cellular elements. A second series of components has now to be discussed—the chromidial granulations. These are so named in the present study because they are defined as having nuclear or chromatinic composition, to distinguish them from other granules which are certainly cytoplasmic and nonspecific. Those chromidia pervade the tissue. They are intermediate between the histologic cancer unit and the actual unit.

Table I includes a list of these components of the cancer unit.

TABLE I

THREE GROUPS OF CHROMIDIAL GRANULES AND INCLUSION BODIES

I. *Granular forms.* These occur in the intercellular tissue and in the connective tissue spaces as well as within tumor cells.
- (a) Submicroscopic: visible by dark-field method (elementary bodies).
- (b) Microgranules: up to 1μ in diameter. As these often show a halo, they constitute the smallest "inclusion bodies."
- (c) Macrogranules: (from 1 to 3μ).
- (d) Both (b) and (c) in the same tissue (Figs. 3, 4).
- (e) Packets of (usually) eight particles, sometimes found within an oval deeply oxyphile sharply contoured "cell." These bodies are both trachy- and pachychromatic (Fig. 3).

II. *Cell inclusions in the usual sense.*
- (a) Lying within signet-ring cells. The classical Plimmer bodies belong here. Blastomyces have a similar appearance (Figs. 5, 6).
- (b) Smaller bodies, resembling coccidia.
- (c) Metachromatic masses within tumor cells, suggestive of plasmodial bodies; therefore, resembling protozoa.
- (d) Pachychromatic oval bodies resembling torulae or yeasts.

III. *Filamentous forms.* These are (a) noded, (b) spirillar or wavy in form. They are located in mononuclear leucocytes, in the circulating blood, and also occur in tumor cells—usually in the cytoplasm, sometimes in the nucleus. When multiple and intertwined, as may occur in some cases of advanced carcinoma, they resemble a small mycelium.

All these bodies are stainable with dyes, but all are not visible in histologic preparations. Some of them (I-a) are only recognizable by means of dark-field illumination or by means of the Zeiss polychromar, and only in the freshly obtained tumor juice or "milk." These include innumerable extremely minute granulations in a state of highly active movement. Others are only seen in the fresh blood from a cancer patient, though with difficulty because of their only momentarily coming into view from the depths of the specimen; the

larger bodies present distinctive (vermicular or undulating) movements. Others are only seen with certainty within the blood cells (monocytes) in Leishman preparations.

The Shape of Granulations in Stained Preparations.—Those of the smallest size are spherical. Even the smallest may be enclosed in a clear halo like the cell inclusions in various virus diseases. Other forms are dumbbells, "haltères," oval forms with lateral buds, pyriform shapes, beaded forms, irregular forms, and filamentous forms. Some of these are exactly like wandering leucocytes seen among the epidermal cells of an epithelioma; some resemble yeasts. Mulberry forms suggest autogenous multiplication. Bizarre shapes, indicative of

Fig. 5.—Free-swimming tumor cell with contained Plimmer body. Vital staining with neutral red.

Fig. 6.—Large carcinoma cell containing Plimmer body in a state of division with free chromidial granules.

pseudopods, suggest livingness (fresh preparations). Irregularly quadrilateral shapes in the wet specimen, with refractile granules in them, exhibit motility. Octets whose components are pyriform suggest a reproductive form (ascospores), though they are generally passed over as caryorrhectic and pycnotic leucocytes, or as phagocyted matter. Forms usually described as mitochondria may occur, among which are filamentous forms marked with nodal thickenings. (These can also be demonstrated in blood cultures.) The larger inclusion bodies, up to the size of Plimmer bodies, are also spherical, and usually present a halo.

Plimmer bodies are oval, vesicular, or rounded, sometimes hyaline bodies within large cancer cells, the host-cell nucleus being the same size as the in-

clusion, but usually of crescentic shape. Within the body occurs a spherical deeply staining mass of relatively small size. Fig. 5 shows one of these bodies in a wet preparation, vitally stained with neutral red. The presence of definite nuclear structure is demonstrated in Fig. 6, and shows that these bodies are not degenerative products.

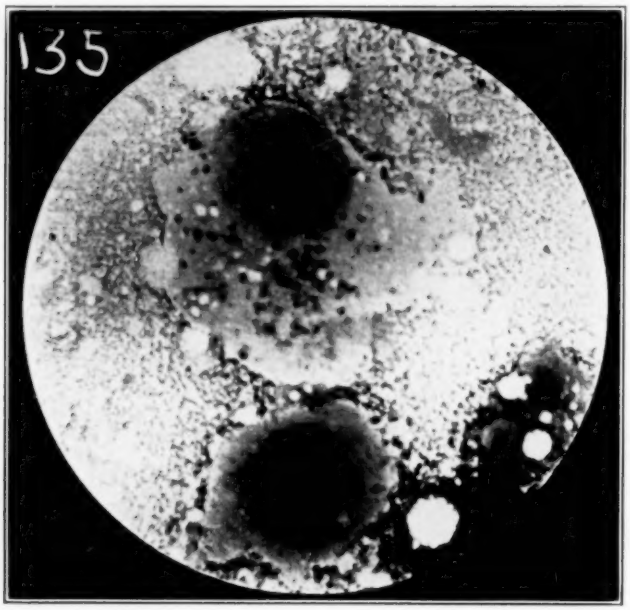


Fig. 7.—Large carcinoma cell containing numerous "virus bodies" or macrogranules. Smear of fresh operation material. Leishman stain. The background is stippled with microgranules of varying size, which include also elementary bodies only seen with dark-field observation.

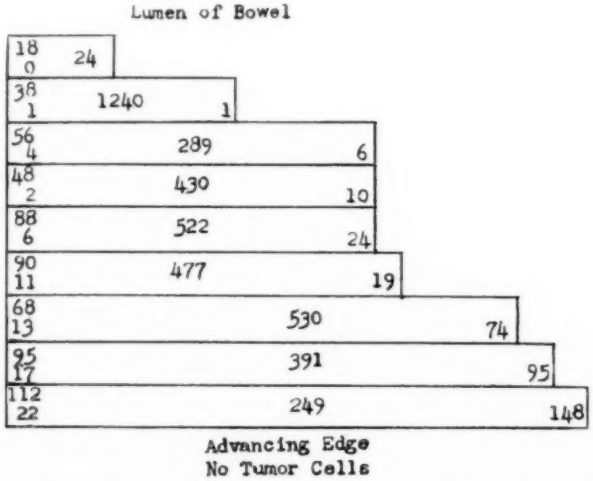


Fig. 8.—Edge of an adenocarcinomatous ulcer. Chromidial analysis of the tissue. Each horizontal line marks off a high-power field of the section. The horizontal lengths are proportional to the number of fields occupied by tumor tissue at the respective levels.

Numerals in the rectangles: left top corner is the number of microgranules, left lower corner is the number of signet-ring cells. Right hand lower corner gives the number of hyperchromic nuclei. The large number in the middle of each rectangle gives the number of macrogranules.

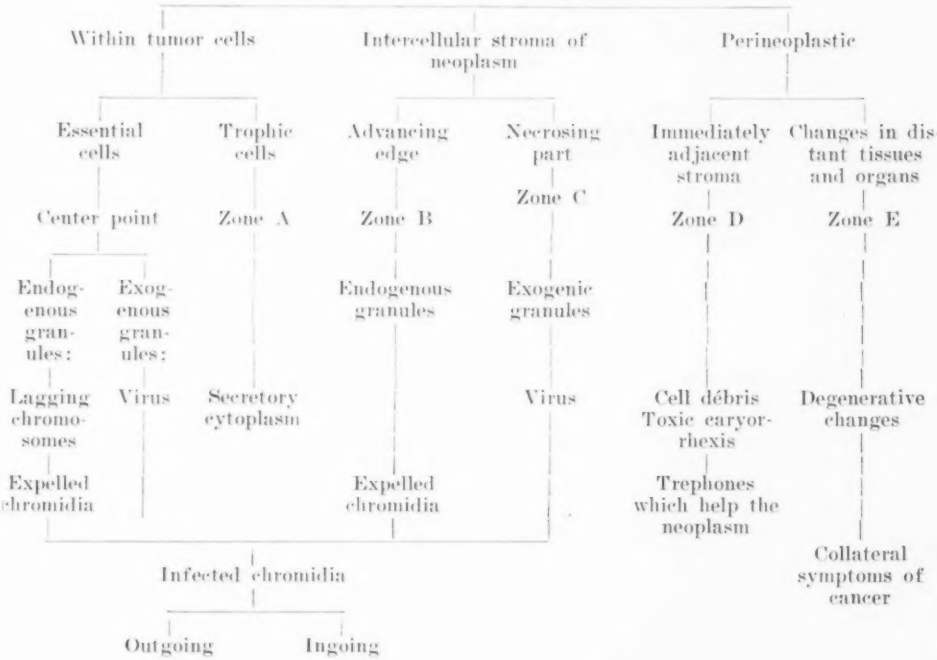
CORRELATION OF CELLS WITH GRANULATIONS

Having listed all the elements which can be found in tumor tissue of whatever kind, we proceed to place them in position. First, a plan of the tissue is drawn up to show a low power magnification and thus assign an orientation—division into districts according to histoanatomic structure and to the position of the several cell types. The several kinds of chromidia are now placed, and the pattern formed is thus made evident. They show focal groupings, zonal groupings, or diffuse scattering. Some are intracellular, either in the resting or in the dividing cell; others are intercellular. Some of the latter are between the tumor cells themselves, others are in the necrotic portions or in the duct lumina.

Thus, Fig. 8 shows the actual numbers of granules of various kinds in the plane of one section as met with in successive histoanatomic regions. This correlation is more clearly brought out in the accompanying schema, which assigns a particular place and function to the chromidia.

TABLE II

HISTOLOGIC RELATIONSHIPS OF CHROMIDIAL GRANULES



The tumor cells are not only static, they are also dynamic, and, therefore, a place has to be found in the scheme for the occurrence of mitosis, especially as one would naturally expect chromidia to show a relation to cell division, especially when mitosis is of pathologic form. They are, therefore, looked for in mitosing cells—whether normal or pathologic. The most important study of pathologic mitosis is that recorded by Politzer,[3] that of the effect of x-rays

on the cells. The fact that chromatolysis occurs under such conditions, is the argument for saying that the chromidia are merely and solely degeneration products.

The chief importance of correlating chromidialization with mitosis is that it provides a point of time from which to measure the events in the tissue. For, if the various chromidial forms are chronologically related, it is necessary also to ascertain how they fit in with the changes continuously proceeding within the tumor cells. Just as the chromidia culminate in a particular type so the tumor cells culminate in a particular form of structure. If the chromidia favor dividing cells, it is clear that every cell of a neoplasm is not equally "malignant," meaning by malignancy "ability to harbor and nourish virus." That is, only virus-containing cells are malignant at any given time.

Hence, in assigning a dual nature to malignancy, the power of multiplication cannot be restricted to the tumor cells alone. The chromidia evidently multiply also, and though they are apparently fixed or stable objects, without biologic organization, their varying sizes may correspond with differences of age. As with all biologic entities, a cycle of development is conceivable, the most minute granules being at one end of the scale and the Plimmer bodies at the other. The various bodies listed in Table I provide a means of "typing" cancerous tissue, and a number can be assigned to each case according to the kind of body which appears furthest on in the list. Thus, when "octets" are found, even in small numbers, the type would be recorded as "Ie," rather than "Ib," "Ic," or "Id"; and if inclusions in signet-ring cells are present, the type is called "II."

The successive types represent a progression toward maturation of the cancerous process, though every neoplasm is not inevitably going through the whole series. Further, the chromidia in some specimens of cancer suggest that an alternation of forms may occur, and this implies the possibility of some of the bodies being dioecious (or multisexual) in nature. (Thus, octets would correspond to ascosporangia.)

But the cycle applies to cancer only in a collective sense, because it only emerges from an analysis of a succession of cases, and not from repeated

TABLE III

ANALYSIS OF CASES ACCORDING TO SITE OF PRIMARY, SHOWING THE FREQUENCY OF OCCURRENCE OF THE VARIOUS GRANULE TYPES

	TYPE I					TYPE II				TYPE III		TOTAL
	a	b	c	d	e	a	b	c	d	a	b	
External and squamous carcinoma (face, mouth, throat, etc.)	2	3	7	7	1	9	1	11		18	1	59
Uterine epithelioma		7	6	9		7		2	2	25	14	17
Gastric		1	6	4	2	8		1	1	13	3	39
Intestinal	2	5	6	1	6	13		1	6	7	8	51
Breast	7	4	9	7	2	30	1	7	1	10		76
Sarcoma	1	4	1	2		3		2		4	3	19
Lung		2		2	1	2				8	6	20
Unclassified sites		2	1			4				10	1	13
Total	12	28	36	32	12	76	2	24	10	95	36	349

biopsies of single cases. In the series analyzed in Table III, the II type, for instance, is rare in sarcoma and some forms of carcinoma.

To conclude, the complete interpretation of the cancerous tissue entails superposing the several phases of chromidia upon the successive histologic loci. The abundance or paucity of the various types is equally important in forming an opinion as to the degree or severity of the disease and the biologic stage to which it has reached.

DISCUSSION

The evidence available in favor of the suggested method of interpreting the histologic structure of neoplasms can only be referred to briefly within the limits of this paper.

Reduced to strict essentials, the granulations may be (1) artifacts or adventitious matter; (2) normal cell structures or cell products; (3) pathologic cell products and by products, that is, degenerative in nature, pathologic mitoses; (4) bacterial contaminations or secondary infections. The need to exclude the first item is most evident when studying material by dark-ground illumination. The second and fourth items are not difficult to eliminate. Hence the real problem is the third—whether the chromidial granules are simply degenerative in origin and not causally related to the disease. The arguments customarily raised against a causal relationship may be passed over, because they are repeatedly and unfailingly brought forward in the literature. There are nevertheless very good reasons for advocating an opposite conclusion. Among them are the following:

Histologic: (1) The fact that host cells and cell fragments can be mimicked by foreign cells and organisms. Recognition of this possibility underlies the repeated efforts to devise differential staining methods (with or without optical devices) for distinguishing with certainty two otherwise exactly similar objects. (2) The demonstration that if a dilute culture of cryptomyces is admixed with a fresh preparation of normal blood, it can be very difficult to discover the presence of the organism; similarly, that a histologic section of ascomycete perithecium can present a very close resemblance to the tissue of round-celled sarcoma within restricted microscopic fields. (3) As a practical fact, eminent pathologists insist that even a cancer cell is indistinguishable from an ordinary body cell (this being an argument that the cancer cell is necessarily always a host cell). (4) Appearances alone will not rule out the possibility of some chromidia in a cell being infective. (5) The "octets" of Table I—eight sharply defined refractile bodies of oval or pyriform shape—are not a feature of the ordinary course of leucocyte caryorrhexis. (6) Divisions and mitoses can be seen in the signet-ring cell inclusions. (7) Experimental inoculations with cultures of cryptomyces produce bodies exactly similar to those under discussion, and in this case the "chromidia" are definitely identical with phases of the injected organisms. (8) Diagnosis that a biopsy is cancerous solely on the basis of the presence of these bodies has been proved correct by the subsequent history, when the disease finally became clinically and histologically established.

Clinical: (1) Tumors showing "degeneration" are more malignant than those which retain well-formed structure throughout; in other words, the more numerous the granulations, the more rapid the spread of the disease. (2) The evidence of Table III, though this needs supplementing by an exact analysis of the after history in every case therein, in order to show the higher types as clinically more malignant or associated with a wider dissemination through the body. Actually, however, this correlation is not uniform, being affected partly by the patient's reaction and partly by the previous medical history. As was discussed in regard to goiter, several diverse factors contribute to the picture. (3) Correlation of blood picture with chromidial types. Discrepancies are found to occur here because of the effect of concurrent treatment. The inclusion bodies disappear completely under radium therapy, remaining absent for a long period of time; x-ray therapy has a similar effect, but of

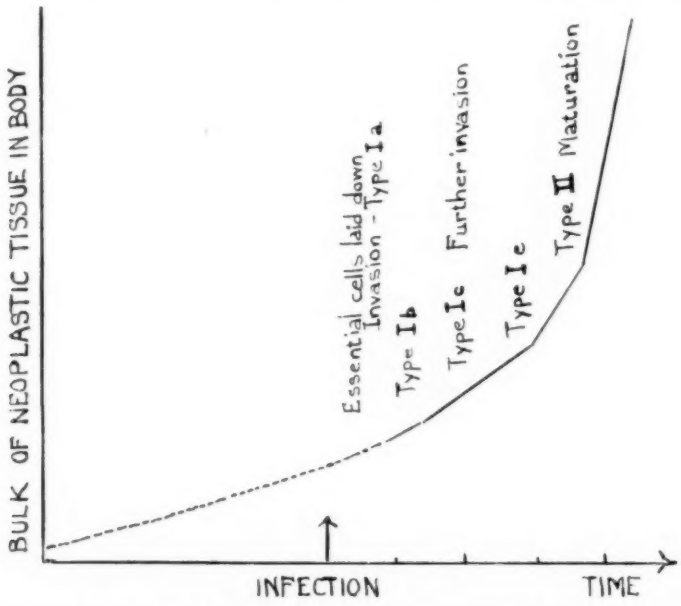


Fig. 9.—Relation between rate of progress of cancer and type of chromidialization. The rise in the curve represents schematically the increasing bulk of neoplastic tissue in the body.

only a few weeks' duration. (4) Biochemical data: determinations of the pH of the blood, of blood calcium, and of glucose tolerance do not give uniform results in all kinds of cancer. This can be explained on the basis of the histologic factors. Thus, dark-field study of fresh tissue shows abundance of fatty acid (anisotropic crystals) and the presence of crystals of calcium oxalate in some tumors and not in others. (It is suggestive that both these findings are also a feature of fungal cultures.) Neoplasms may also be described as being sometimes open, sometimes closed, according to the degree of fibrosis, or blocking of vascular channels by columns of tumor cells, or abundance of chromidial formation. Certainly the blood picture shows abeyance of the distinctive changes ("quiescence") when the tissue also suggests a "closed" form. (5) The time factor. This presents three relations: (a) the stage of

the disease, (b) the duration of the successive chromidial phases, (c) the incidence of the important precancerous disease (i.e., chronologically, not etiologically precancerous). In some cases the clinical history shows a succession of periods with which the chromidial types also correspond (Fig. 9). Blood studies bring this relation out best, because it is rarely possible to observe the tissue at different periods in one case. The time curve is relative, not absolute; the lengths of the abscissae on the chart bear proportional values only. As to (c), the previous medical history, the date and duration of each important illness prior to the first signs of the neoplasm are recorded in order to mark out the intervals (in weeks or months) between each pair of events. The interval between the first sign of neoplasm and the first recurrence has a definite value (Douglas Webster[4]). These intervals are explained by the histologic structure, as the chromidia also take time for their evolution, even if they only pertain to the constitutional factor underlying the disease. (6) The relation to choice of treatment. The ultimate fact about treatment is the choice between surgery or radiation, or both, which in itself tacitly admits the disease to be local at the onset. Up to a certain point, the local lesion or "primary" is the whole disease, and if it could be completely removed, the case would be definitely "cured." The unwillingness to speak of a cure is the permanent doubt whether removal was complete. The present research points to the conclusion that by the time the tumor is there, the blood already shows the characteristic evidences of virus infection. Hence it is relatively unimportant whether the neoplasm is to be regarded as the factory of the virus (i.e., primarily a local disease to start with) or only its storehouse or granary (i.e., a constitutional disease with local manifestation). In either case local treatment is not alone sufficient to ensure a lasting cure, and cases occur in which local radiation forces the chromidia further on, or into the blood and internal organs. In such cases, the inclusion bodies in the blood are found still present, despite the absence of any other evidence of the disease. This supports the argument that cancer is not just "neoplasm," but a disease requiring general treatment as well.

SUMMARY

A method of interpretation is presented which furnishes a means of correlating the structure of neoplasms with their behavior and with the clinical features of the cancer disease.

The essential basis of the interpretation is that there is a dualism of nature in the neoplasms—one component being the cellular one, and the other the chromidial granules. The tumor cells are partly a reaction to virus infection, partly the site of proliferation of the virus, so that only the infected cells are the really malignant ones.

The chromidial granules are not considered to be degeneration products, but inherent in the propagation of the virus. They are thus raised to a position of special significance.

A special analysis of cases studied according to the types of granulation is presented, the associated researches forming the ultimate basis of the present thesis.

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TOXICITY, THERAPEUTIC ACTIVITY, AND MODE OF ACTION OF SULFANILAMIDE IN EXPERIMENTAL STREPTOCOCCUS INFECTIONS OF RABBITS*

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SINCE septicemia of human beings due to beta hemolytic streptococci is usually the result of a rapid overflow of organisms into the blood from a primary focus of infection of the fixed tissues which cannot be effectively balanced by the clearing mechanism, we have employed rabbits in this investigation inoculated intradermally and intravenously with *virulent* streptococcus of this type, with the production of severe local skin lesions, septicemia, and secondary suppurative arthritis, according to the method of Kolmer and Rule.[1] These lesions not only permit direct clinical observations but likewise and more importantly, bacteriologic examinations of the skin, blood, and joints as a means for ascertaining disinfection of these tissues by chemical agents, and in these respects constituting a more satisfactory experimental disease than experimental streptococcus infections of mice so commonly employed.

In a previous communication by Kolmer, Brown, Raiziss, Rule, and Clemence,[2] it was reported that prontosil soluble, while exerting some curative effects upon the local skin lesions, failed to produce complete disinfection in the dose employed (0.015 gm. per kg. daily for five days in succession), being in both respects less effective than pyridyl sulfide in similar dosage per body weight. Both compounds also exerted some bactericidal effects upon the associated septicemia, but were practically ineffective in the treatment of the secondary suppurative arthritis in the doses employed.

The present investigation was confined entirely to a study of sulfanilamide† in the treatment of these experimental lesions, since its lower toxicity has permitted the administration of much larger doses. Furthermore, it has been pos-

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sible to determine quantitatively the concentration of free and conjugated sulfanilamide in the blood in relation to toxicity and therapeutic effectiveness, as well as of the compound in the local skin lesions in relation to disinfection, as determined by clinical observations and bacteriologic examinations.

Furthermore, while the maximum tolerated and minimal lethal doses of sulfanilamide and its derivatives for the lower animals per kilogram of weight by different routes of administration have been thoroughly studied by various investigators, its chemopathologic effects have not been reported upon, so that we have included this important phase of the subject in the present investigation.

TOXICITY OF SULFANILAMIDE IN RELATION TO CONCENTRATION IN THE BLOOD OF NORMAL AND NEPHRITIC RABBITS

With the particular brand of sulfanilamide employed, Raiziss, Severac, and Moetsch[3] have reported the maximum *single* tolerated dose for mice by subcutaneous injection to be about 2 gm. per kg. of weight. By oral administration to rabbits the maximum *single* tolerated dose was about 1.5 gm. per kg., since 94 per cent of animals survived the administration of this amount.

In our experiments rabbits have lived indefinitely following ten daily *subcutaneous* injections of 0.004 to as much as 0.160 gm. per kg. (corresponding to 9.6 gm. per 60 kg.), each dose being divided into two portions, given six hours apart.

By *oral* administration the maximum tolerated dose was between 0.2 and 1.0 gm. per kg. daily, divided into two doses six hours apart, as an animal survived after six daily doses of the latter, a third animal succumbing after three daily doses of 2.0 gm. per kg.

Quantitative analyses of the blood for *free* sulfanilamide by the method of Marshall, Emerson, and Cutting[4] were made about twenty-four hours after each daily administration, and because of rapid absorption and elimination, showed none of the compound in the blood for the first one to three days. After this time, however, the compound accumulated faster than it could be eliminated, but even a daily dose of 0.160 gm. per kg. by subcutaneous injection (divided into two doses six hours apart) did not result in a concentration of *free* sulfanilamide over 1.4 mg. per 100 c.c. (ninth day). A daily dose of 0.2 gm. per kg. by oral administration (divided into two doses six hours apart) resulted in a maximum concentration of 0.8 mg. per 100 c.c. (on the ninth day), but a daily dose of 1.0 gm. per kg. by oral administration (divided into two doses six hours apart) produced a concentration of 12.5 mg. per 100 c.c. on the seventh day when this animal succumbed from the toxic effects.

In a second experiment the compound was given orally to four rabbits in a daily total dose of 0.2 to 0.8 gm. per kg. (divided into two doses daily) for five days. One receiving 0.4 gm. per kg. twice daily died after the eighth dose (fourth day), indicating that about 0.3 gm. twice a day by oral administration was about the maximum tolerated dose by this route and manner of administration.

Free and conjugated sulfanilamide (para-acetylaminobenzenesulfonamide) was determined in the blood of each animal three hours after the first and

seventeen hours after the second dose each day by the method of Marshall.[5] Because of rapid absorption, maximum amounts were found in the blood as early as three hours after the first dose in each animal. It was noted that two daily doses of 0.1 and 0.2 gm. per kg. by oral administration did not result in a concentration of *free* sulfanilamide higher than 6.5 mg. per 100 c.c. of blood, whereas it required the maximum tolerated dose of 0.3 gm. per kg. twice daily before a concentration of 10 to 14 mg. per 100 c.c. resulted. This is believed to be the concentration required for pronounced therapeutic effects. A twice daily dose of 0.4 gm. per kg., which was lethal on the fourth day, resulted in a maximum concentration of 10.6 mg. per 100 c.c. three hours after the fifth dose (third day), the animal succumbing after the eighth dose (fourth day) with 6.5 mg. per 100 c.c.

The blood concentration of the conjugated or acetyl compound, the production of which may be a defensive mechanism of detoxification, varied from 1.4 mg. per 100 c.c. in the case of a rabbit receiving 0.1 gm. per kg. twice daily to as high as 29 mg. in the case of a rabbit receiving 0.4 gm. per kg. twice daily, but the therapeutic effectiveness of this conjugated compound is still uncertain.* If it contributes to therapeutic effectiveness, the *total* sulfanilamide in the blood of rabbits has varied from 10 mg. per 100 c.c. in the case of 0.1 gm. per kg. twice daily to as much as 37 mg. when 0.4 gm. per kg. is given twice daily by oral administration.

In a third experiment six rabbits were given an intravenous injection of mercurophen in dose of 0.002 gm. per kg. for the purpose of producing a mercurial nephritis.† Four days later the blood urea nitrogen varied from 60 to 80 mg. per 100 c.c., indicating well-marked nitrogen retention from the kidney injury. Two controls died of this nephritis in thirteen to fifteen days.

The four remaining animals were given sulfanilamide twice daily in dose of 0.1 to 0.4 gm. per kg. by oral administration, beginning three days after the injection of mercurophen. Each daily dose was divided in two, given six hours apart.

Because of the nephritis, the maximum tolerated dose was reduced from 0.3 to 0.2 gm. twice daily per kg., presumably because of interference with the elimination of the compound. In confirmation of the observations of Marshall and his colleagues,[4] it was observed that the nephritis resulted in the accumulation of both free and conjugated sulfanilamide in marked degree as compared with normal rabbits; also that toxic effects were produced by a smaller amount of free sulfanilamide in the blood of nephritic than in normal rabbits and indicating the need for extra caution in dosage for human beings with nephritis. For example, the dose of 0.1 gm. per kg. twice daily resulted in a maximum concentration of 5.5 mg. of free and 4.5 mg. of conjugated sulfanilamide per 100 c.c. of blood in the case of a normal rabbit, but of 9.2 mg. of free and 21.2 mg. of conjugated in the case of a nephritic rabbit; a dose of 0.4 gm. per kg. twice daily resulted in a maximum concentration of 12 mg. of free and 29 mg. of conjugated

*Dr. George W. Raiziss informs us (personal communication) that he believes it to be therapeutically effective in streptococcus septicemia of mice.

†Histologic examination has shown the production of severe tubular nephritis (nephrosis) characterized by degenerative and desquamative changes in the tubular epithelium with calcareous infiltration.

sulfanilamide per 100 c.c. of blood in the case of a normal rabbit, but of 22 mg. of free and 38 mg. of conjugated in a nephritic rabbit.

In the normal rabbit it was found that when a twenty-four-hour level of free sulfanilamide, amounting to 10 mg. per 100 c.c., was maintained for a few days, death ensued. This level was only attained when the enormous dose of 0.4 gm. per kg. was administered twice daily and then only after elimination had begun to be impaired. In other words, it would appear that attempts to attain a blood level of about 10 mg. per 100 c.c. during clinical treatment may be hazardous for human beings unless they have a greater tolerance for sulfanilamide than rabbits. Attempts to reach such levels may account for the appearance of the toxic effects reported in the literature.

In an additional experiment the blood concentration of free and conjugated sulfanilamide was determined in a normal and a nephritic rabbit receiving a single dose of 0.3 gm. sulfanilamide per day per kilogram of weight. The latter showed a blood urea nitrogen of 54 mg. per 100 c.c. three days after the injection of mercurophen, when the administration of sulfanilamide was begun.

It was observed that there was a very rapid absorption of sulfanilamide from the gastrointestinal tract, about 50 per cent of the total free sulfanilamide being found in the blood in the first half hour. This applied to both the normal and nephritic animals and in both the maximum amounts of free sulfanilamide were found in from two to three hours. In the normal rabbit there was a decrease in free sulfanilamide to negligible amounts in thirty-two hours, while the nephritic rabbit showed a somewhat delayed elimination. In thirty-two hours there was still 2.5 mg. free sulfanilamide as compared to 0.9 mg. per 100 c.c. in the normal animal.

This difference was much more definite when the curves of conjugated sulfanilamide were considered. Here it was found that a considerable accumulation of the conjugated compound occurred in the nephritic rabbit, equal in amount to the free sulfanilamide, although the peak of this curve was delayed several hours over that of the free sulfanilamide. The conjugated sulfanilamide found in the normal rabbit reached a maximum value somewhat earlier and was only about one-half that of the free sulfanilamide. Little or no conjugated sulfanilamide was detectable in the first half hour, although, as has been indicated, considerable free sulfanilamide already appeared in the blood at this time.

These differences point to an interesting speculation as to the mechanism of in vivo conjugation. Combined sulfanilamide must be formed either in the gastrointestinal tract and be absorbed and eliminated more slowly, or it is actually conjugated during passage through the fixed tissues. Retarded elimination is indicated in both the normal and nephritic animal; more so in the latter since general elimination is definitely impaired. Slower absorption of conjugated than of free sulfanilamide may account for the delayed appearance of the former if it is formed in the gastrointestinal tract, but such a mechanism does not account for the greatly increased conjugated sulfanilamide in the nephritic rabbit. This might be due to a selective kidney elimination, but defective elimination should also bring about a great increase of free sulfanilamide

TABLE I

EFFECT OF SULFANILAMIDE UPON LOCAL STREPTOCOCCUS SKIN LESIONS OF RABBITS*

NO.	DAILY DOSE PER KG.** (GM.)	ROUTE OF ADMIN.	FIRST DAY BEFORE TREATMENT		SECOND DAY		THIRD DAY		FOURTH DAY		FIFTH DAY		SEVENTH DAY		TENTH DAY	
			*** CLINICAL	CULTURE	CLIN.	CULT.	CLIN.	CULT.	CLIN.	CULT.	CLIN.	CULT.	CLIN.	CULT.	CLIN.	CULT.
1	0.004	Subcut.	4	+	4	+	2	+	1	+	Absc.	+	Absc.	+	Absc.	+
2	0.008	Subcut.	4	+	4	+	2	+	1	+	1	+	Absc.	+	Died	
3	0.08	Subcut.	4	+	4	+	2	+	1	+	1	+	Absc.	+	Absc.	+
4	0.2	Orally	4	+	4	+	3	+	1	+	1	+	Absc.	+	Absc.	+
5	0.5	Orally	4	+	4	+	3	+	3	+	3	+	Absc.	+	Absc.	+
6	1.0	Orally	4	+	4	+	3	+	Died							
7	Control	–	4	+	4	+	4	+	4	+	4	+	Absc.	+	Died	
8	Control	–	4	+	4	+	4	+	4	+	3	+	2	+	Absc.	+

*Rabbits inoculated intradermally with 0.6 c.c. of eighteen-hour hormone broth culture.
**Divided into two doses six hours apart daily for five days; first dose given twenty-four hours after inoculation.
***4 = severe lesion; 3 = less severe, etc., absc. = abscess.

since the doses were the same. The fact that the free sulfanilamide remains about the same in both animals, despite decreased elimination in the nephritic rabbit and the greatly increased combined sulfanilamide in the latter, can be explained if it is assumed that conjugation takes place while circulating through the fixed tissues. The latter site of conjugation would tend to depress the maximum values found for free sulfanilamide despite decreased elimination because of continual conjugation, and would cause a corresponding increase as well as a somewhat delayed appearance of combined sulfanilamide, a condition actually found to exist.

HISTOLOGIC EVIDENCES OF TOXICITY OF SULFANILAMIDE

All of the animals given ten daily doses of 0.004 to 0.160 gm. per kg. subcutaneously and 0.2 to 2.0 gm. per kg. orally were sacrificed after the tenth dose of the compound, and the kidneys, liver, spleen, adrenal glands, heart, and lungs examined histologically for evidences of injury, as this has been found advisable in chemotherapeutic research instead of depending solely on the duration of the lives of experimental animals for estimating the toxicity of chemical agents.[6] The same organs were submitted to histologic examination from the two rabbits succumbing to the oral administration of the compound.

The results were essentially negative and confirmatory of the remarkably low toxicity of sulfanilamide. None showed any histologic evidences of injury in the heart, lungs, adrenal glands, or spleen. The kidneys of a rabbit, given ten daily doses of 0.160 gm. per kg. by subcutaneous injection, showed slight degrees of cloudy swelling of the epithelium of the straight tubules as likewise those given 1.0 and 2.0 gm. orally per kilogram of weight and succumbing on the eighth and fourth days, respectively. The liver of a rabbit receiving ten daily doses of 0.160 gm. subcutaneously per kilogram and that of the rabbit given three daily doses of 2.0 gm. by oral administration likewise showed marked cloudy swelling of the lobular epithelium, particularly well marked about the central veins; we are not at all sure that these changes were produced by sulfanilamide, since they have been found in control rabbits and those subjected to streptococcus infection.

EFFECT OF SULFANILAMIDE IN THE TREATMENT OF STREPTOCOCCUS INFECTIONS OF RABBITS

In one experiment ten rabbits were given an injection of 0.6 c.c. of eighteen-hour hormone broth culture of virulent hemolytic streptococcus in the abdominal skin. Severe local lesions yielding positive cultures resulted in twenty-four hours, at which time treatment with sulfanilamide was begun on eight of the animals, the doses per kilogram and routes of administration being shown in Table I. Two were kept as untreated controls.

It will be observed that all receiving 0.004 to 0.08 gm. subcutaneously and 0.2 to 1.0 gm. orally per kilogram of weight daily for five days in succession, showed well-marked clinical effects as compared with the untreated controls, but positive cultures of the lesions were secured throughout a period of ten days,

indicating incomplete disinfection of these local lesions. Two of the treated and one of the control animals succumbed, and the balance developed abscesses, five to seven days after inoculation.

In a second experiment eighteen rabbits were inoculated intradermally with the same amount of culture and at the same time given 0.5 c.c. intravenously. Twelve were subjected to treatment, the doses and routes of administration being given in Table II, while six were kept as untreated controls. The primary purpose of this experiment was to determine the effects of sulfanilamide on the prevention of suppurative arthritis.

TABLE II

EFFECT OF SULFANILAMIDE UPON THE LOCAL SKIN LESIONS AND IN THE PREVENTION OF ARTHRITIS OF RABBITS*

NO.	DAILY DOSE PER KG.** (G.M.)	ROUTE OF ADMIN.	24 HR. AFTER INOCULATION; LOCAL LESIONS***	LOCAL SKIN LESIONS	EFFECT OF TREATMENT IN PREVENTION OF SUPPURATIVE ARTHRITIS
1	0.004	Subcut.	2	Regressed; died 7 days	Developed (6 days)
2	0.004	Subcut.	2	Regressed; died 7 days	Developed (6 days)
3	0.008	Subcut.	3	Healed 8 days; survived	Absent
4	0.008	Subcut.	2	Healed 9 days; survived	Developed (7 days)
5	0.08	Subcut.	3	Healed 6 days; survived	Absent
6	0.08	Subcut.	4	Healed 6 days; died 10 days	Developed (6 days)
7	0.2	Orally	3	Healed 7 days; survived	Absent
8	0.2	Orally	2	Healed 6 days; survived	Developed (8 days)
9	0.5	Orally	2	Healed 6 days; survived	Absent
10	0.5	Orally	2	Regressed; died 10 days	Developed (8 days)
11	1.0	Orally	2	Regressed; died 6 days	Absent
12	1.0	Orally	3	Regressed; died 13 days	Absent
13	Control	–	3	Progressed; died 6 days	Developed (6 days)
14	Control	–	3	Progressed; died 15 days	Developed (7 days)
15	Control	–	2	Regressed; survived	Absent
16	Control	–	2	Progressed; died 6 days	Developed (6 days)
17	Control	–	1	Progressed; died 7 days	Developed (6 days)
18	Control	–	3	Regressed; survived	Developed (7 days)

*Rabbits inoculated with 0.6 c.c. of eighteen-hour hormone broth culture intradermally and 0.5 c.c. intravenously.

**Divided into two doses six hours apart daily for five days; first dose given twenty-four hours after inoculation.

***4 = severe lesion; 3 = less severe, etc.

Of the six untreated controls the local lesions progressed in five, and all but one, or 83 per cent, developed arthritis; four of the animals succumbed.

Of the twelve animals in which treatment was started twenty-four hours after inoculation, consisting of five daily doses of 0.004 to 0.08 gm. subcutaneously and 0.2 to 1.0 gm. orally per kilogram of weight, the local lesions regressed or healed in all; six, or 50 per cent, developed arthritis, and six, or 50 per cent, succumbed.

In a third experiment a series of rabbits were inoculated intradermally with 0.6 c.c. of eighteen-hour hormone broth culture and given 0.5 c.c. intravenously. One week later fifteen had developed arthritis, involving one to three joints. At this time treatment was started on twelve, while three were kept as controls. Six were given five daily subcutaneous injections of 0.004 to 0.08 gm. per kg. of weight, each amount being divided into two doses, six hours apart.

The purpose of the experiment was to determine the therapeutic effects of sulfanilamide upon the arthritis, and the results are shown in Table III. Joint and blood cultures were made daily.

TABLE III

EFFECT OF SULFANILAMIDE UPON THE TREATMENT OF SUPPURATIVE ARTHRITIS OF RABBITS*

NO.	NUMBER JOINTS DEVELOPING INFECTION 1 WEEK AFTER INOCULATION	DAILY DOSE PER KG.** (GM.)	ROUTE OF ADMIN.	JOINT LESIONS***	BLOOD CULTURES AT TIME OF DEATH
1	2	0.004	Subcut.	Progressed; died 20 days	+
2	3	0.004	Subcut.	Progressed; died 19 days	+
3	3	0.008	Subcut.	Progressed; died 10 days	+
4	3	0.008	Subcut.	Progressed; died 14 days	−
5	2	0.08	Subcut.	Progressed; died 10 days	not made
6	1	0.08	Subcut.	Progressed; died 13 days	+
7	3	0.2	Orally	Progressed; died 14 days	+
8	3	0.2	Orally	Regressed; lived indefinitely	0
9	2	0.5	Orally	Regressed; lived indefinitely	0
10	3	0.5	Orally	Progressed; died 20 days	+
11	1	1.0	Orally	Progressed; died 18 days	−
12	1	1.0	Orally	Regressed; lived indefinitely	0
13	3	Control	0	Progressed; died 10 days	+
14	2	Control	0	Progressed; died 13 days	+
15	3	Control	0	Progressed; died 11 days	+

*Rabbits inoculated with 0.6 c.c. of eighteen-hour hormone broth culture intradermally and 0.5 c.c. intravenously.

**Divided into two doses six hours apart daily for five days; first dose given seven days after inoculation.

***Joint cultures made every two to three days after development of arthritis; all positive.

The arthritis of all three untreated controls progressed, and all three succumbed in ten to thirteen days after inoculation with positive joint and blood cultures.

Of the twelve treated animals, the arthritis continued to progress in nine, or 75 per cent; nine, or 75 per cent, succumbed with positive blood and joint cultures. Arthritis regressed in three (25 per cent), and the animals lived indefinitely, although positive joint cultures persisted throughout a period of twenty-one days following inoculation.

EFFECT OF SULFANILAMIDE UPON PHAGOCYTOSIS AND CONCENTRATION OF THE COMPOUND IN THE BLOOD AND LOCAL LESIONS OF RABBITS

In one experiment six rabbits were inoculated intradermally with 0.6 c.c. of eighteen-hour hormone broth culture. Twenty-four hours later severe local lesions had developed and smears showed that from 12 to 25 per cent of the polymorphonuclear leucocytes had become phagocytic.

Three of the animals were now given 0.004 to 0.08 gm. sulfanilamide by subcutaneous injection, and two 0.1 and 0.5 gm. by oral administration per kilogram of weight, each dose being divided into two portions and given six hours apart (Table IV). One animal was kept as an untreated control.

Twenty-four hours later the local lesions of four of the five treated animals showed clinical regression, and five showed an appreciable degree of increased phagocytosis by the polymorphonuclear leucocytes of the inflammatory exudates.

TABLE IV

EFFECT OF SULFANILAMIDE UPON PHAGOCYTOSIS AND CONCENTRATION OF THE COMPOUND IN THE BLOOD AND LOCAL LESIONS*

NO.	LESION 24 HOURS AFTER INOCULATION			TREATMENT****		LESION 24 HOURS LATER			SULFANILAMIDE; MG. PER 100 C.C.	
	CLINICAL	CULTURE	PHAGOCYTOSIS***	DOSE PER KG. (GM.)	ROUTE ADMIN.	CLINICAL	CULTURE	PHAGOCYTOSIS***	BLOOD	LESION
1	4**	+	12	0.004	Subcut.	2**	+	16	0.5	Trace*****
2	4	+	12	0.008	Subcut.	2	+	25	0.2	Trace
3	3	+	25	0.08	Subcut.	4	+	26	0.2	Trace
4	4	+	12	0.1	Orally	3	+	12	0.3	Negative
5	4	+	12	0.5	Orally	3	+	18	0.8	1.3
6	4	+	16	Control	–	4	+	12	Negative	Negative

*Rabbits inoculated with 0.6 c.c. of eighteen-hour hormone broth culture intradermally.
**4 = severe lesion; 3 = less severe, etc.
***Percentage of polymorphonuclear leucocytes in lesion smears found phagocytic.
****Divided into two doses six hours apart.
*****Less than 0.1 mg per 100 c.c.

Free sulfanilamide was found in the blood, varying from 0.2 to 0.8 mg. per 100 c.c.

The lesions were now excised, weighed, ground with washed sand, and extracted in an incubator at 37° C. for twenty-four hours with 95 per cent alcohol. After paper filtration, an aliquot portion of each filtrate was tested for free sulfanilamide. The lesions of three of the treated animals showed traces of free sulfanilamide (less than 0.1 mg. per 100 gm.); one was negative, and one showed 1.3 mg. per 100 gm. (rabbit No. 5 received 0.5 gm. orally per kilogram of weight).

Smears of the local lesions were made twenty-four hours after inoculation in the case of the 8 rabbits listed in Table V. From 4 to 28 per cent of the polymorphonuclear leucocytes were found phagocytic.

TABLE V

EFFECT OF SULFANILAMIDE UPON PHAGOCYTOSIS OF STREPTOCOCCI IN LOCAL SKIN LESIONS OF RABBITS*

NO.	DAILY DOSE PER KG.** (GM.)	ROUTE OF ADMIN.	PER CENT PHAGOCYTIC CELLS IN EXUDATES; DAYS					
			24 HRS. AFTER INOCULATION; BEFORE TREATMENT	2	3	4	5	7	10
1	0.004	Subcut.	10***	48	6	6	16	8	10
2	0.008	Subcut.	8	10	24	36	10	22	Died
3	0.08	Subcut.	12	14	36	38	40	14	14
4	0.2	Orally	16	28	24	8	24	10	14
5	0.5	Orally	4	16	20	32	30	16	12
6	1.0	Orally	20	16	22	Died			
7	Control	–	12	8	14	20	12	8	10
8	Control	–	28	28	26	40	34	8	10

*Rabbits inoculated intradermally with 0.6 c.c. of eighteen-hour hormone broth culture.
**Divided into two doses six hours apart daily for five days.
***Fifty cells counted in smears of lesion; percentage phagocytic.

Three were given subcutaneous injections of 0.004 to 0.08 gm. of sulfanilamide, and three 0.2 to 1.0 gm. by oral administration daily for five days in succession, each amount being divided into two doses, six hours apart. Two were kept as untreated controls.

Phagocytic counts were made daily up to ten days.

In one of the untreated controls, from 8 to 20 per cent of the polymorphonuclear leucocytes were phagocytic throughout, while in the second, from 8 to 40 per cent were phagocytic.

Among five of the six treated animals, the percentage of phagocytes was materially increased during the first one to three days of treatment, but not in the case of No. 5, which was given 1.0 gm. per kg. orally and succumbed three days after inoculation.

Phagocytic studies were also made in the case of all of the eighteen rabbits listed in Table II. As previously stated, these had been inoculated intradermally with 0.6 c.c. of culture, and intravenously with 0.5 c.c.

Six were subjected to treatment with sulfanilamide in dose of 0.004 to 0.08 gm. subcutaneously, and six with 0.2 to 1.0 gm. per kg. by oral administration daily for five days beginning twenty-four hours after inoculation, each amount being divided into two doses, six hours apart. Six animals were kept as untreated controls.

TABLE VI

EFFECT OF SULFANILAMIDE UPON PHAGOCYTOSIS AND CULTURES OF SKIN LESIONS, BLOOD, AND JOINTS OF INFECTED RABBITS*

NO.	DAILY DOSE PER KG.** (GM.)	ROUTE OF ADMIN.	PHAGOCYTOSIS***			LESION CULTURES DAYS					BLOOD CULTURES DAYS					JOINT CULTURES****
			BEFORE	2 DAYS	5 DAYS	BEFORE	2	5	7	10	BEFORE	2	5	7	10	
1	0.004	Subcut.	18	6	0	+	+	–	D		+	+	–	D		+
2	0.004	Subcut.	30	24	4	+	+	–	D		+	–	+	D		+
3	0.008	Subcut.	10	18	0	+	+	+	–	–	+	–	–	–	–	0
4	0.008	Subcut.	32	28	3	+	+	–	–	–	+	+	–	–	–	+
5	0.08	Subcut.	22	8	0	+	+	+	–	–	+	–	–	–	–	0
6	0.08	Subcut.	16	4	0	+	+	–	–	D	+	–	–	D		+
7	0.2	Orally	10	18	2	+	+	–	–	–	+	–	–	–	–	0
8	0.2	Orally	10	6	0	+	+	–	–	–	+	–	–	–	–	+
9	0.5	Orally	30	18	6	+	–	–	–	–	+	+	–	–	–	0
10	0.5	Orally	10	10	0	+	+	+	+	D	+	+	–	D		+
11	1.0	Orally	34	18	0	+	+	D			+	–	D			0
12	1.0	Orally	20	10	0	+	+	–	–	–	+	+	–	+	+	0
13	Control	–	30	11	–	+	+	+	D		+	+	+	D		+
14	Control	–	35	8	0	+	+	+	+	+	+	+	–	+	+	+
15	Control	–	16	16	8	+	+	+	–	–	+	+	+	+	–	0
16	Control	–	16	12	6	+	+	+	D		+	+	+	D		+
17	Control	–	14	12	0	+	+	+	D		+	+	+	D		+
18	Control	–	12	12	4	+	+	+	+	+	+	+	–	+	+	+

*Rabbits inoculated with 0.6 c.c. of eighteen-hour hormone broth culture intradermally and 0.5 c.c. intravenously.

**Divided into two doses six hours apart daily for five days; first dose twenty-four hours after inoculation.

***Percentage of polymorphonuclear leucocytes in lesion smears found phagocytic; 0 = not made.

****Cultures made at same intervals as cultures of lesions and blood; + = positive culture; 0 = no arthritis and culture not made.

Twenty-four hours after inoculation smears of the exudates of the local lesions showed that from 10 to 35 per cent of the polymorphonuclear leucocytes had become phagocytic (Table VI).

Of the six untreated controls, the percentage of phagocytes dropped in the following five days. The local lesion and blood of the one animal (No. 15) became spontaneously sterile on the seventh day, and this animal escaped arthritis and lived indefinitely, but all of the remaining five developed arthritis and showed positive skin lesion, blood, and joint cultures throughout the period of observation, three succumbing about seven days after inoculation.

Of the six receiving five daily subcutaneous injections of sulfanilamide in dose of 0.004 to 0.08 gm., and six from 0.2 to 1.0 gm. by oral administration per kilogram of weight, only two showed an increase of phagocytosis.

Five, or 42 per cent, of the twelve treated animals succumbed as compared to three, or 50 per cent, of the six untreated controls. Ten, or 83 per cent, of the treated animals gave sterile lesion cultures as compared with 17 per cent of the untreated controls; and ten, or 83 per cent, gave sterile blood cultures, although five developed arthritis and four succumbed.

DISCUSSION

These results have confirmed the observations of others in regard to the remarkably low toxicity of sulfanilamide for the normal animal as determined not only by the duration of life and the absence of demonstrable toxic effects, but likewise by the absence of pronounced histologic changes in the various organs following the administration of large amounts per kilogram of weight. They have also shown the rapid absorption of the compound from the subcutaneous tissues and the gastrointestinal tract and its rapid elimination requiring frequent administration for the maintenance of effective amounts in the blood. They have also shown that defective elimination by the kidneys of both free and conjugated sulfanilamide as the result of nephritis materially increases toxicity and indicates smaller dosage and extra caution in the treatment of human beings under these conditions. In other words, sulfanilamide in ordinary dosage does not appear to increase the degree of nephritis, but its toxicity is increased by reason of defective elimination.

Evidence is presented indicating that conjugation of sulfanilamide probably occurs in the blood and the fixed tissues, but the question of its therapeutic properties is uncertain at the present time. If therapeutic effectiveness depends primarily upon free sulfanilamide in the blood, almost toxic and lethal amounts must be given the normal rabbit to attain a concentration of 10 to 12 mg. per 100 c.c., believed to be required in the treatment of human beings.

Our results have shown that sulfanilamide exerts a well-marked clinical effect upon the local skin lesions in rabbits produced by the intradermal injection of virulent beta hemolytic streptococci, but in the doses employed it has only occasionally and irregularly effected complete sterilization of the lesions. It has also demonstrated some effect in the sterilization of the blood and in the prevention of secondary infection of the joints. Probably the duration of treatment employed in these experiments has been insufficient, although the dosage per kilogram of body weight has been in most instances materially greater than

employed in the treatment of human beings. But at all events, it appears that there is need for a compound possessing a greater capacity for disinfection or sterilization of the primary and secondary infections of the fixed tissues than demonstrated by sulfanilamide in these experimental infections of rabbits.

While the mechanism of curative activity of sulfanilamide has not yet been completely elucidated, the results observed in this study tend to confirm the opinion that at least part of its therapeutic effectiveness is due to a promotion of phagocytosis of streptococci in vivo probably by reason of exerting sufficient injury or bacteriostasis of the organisms and destruction of toxins to reduce their antiphagocytic properties. In a recent study of sulfanilamide in the treatment of experimental streptococcus and pneumococcus meningitis of rabbits and monkeys by Kolmer, Rule, and Werner,[7] there was likewise distinct evidence indicating that the compound promoted phagocytosis in the purulent cerebrospinal fluids, and while the amounts of the latter were insufficient for quantitative tests for the presence of the compound, the results indicated that the promotion of phagocytosis was apparently an important phase of therapeutic activity.

SUMMARY AND CONCLUSIONS

1. Sulfanilamide by subcutaneous injection is remarkably low in toxicity for normal rabbits, since five daily doses, as high as 0.160 gm. per kg., were without toxic effects.

2. Ten doses at the rate of two per day by oral administration gave a maximum tolerated dose of about 0.3 gm. per kg. of weight.

3. The compound is also remarkably low in the production of pathologic tissue changes in the normal rabbit. Only the repeated administration of such large amounts as 0.160 gm. subcutaneously and 1.0 to 2.0 gm. orally per kilogram of weight, produced suggestive cloudy swelling of the tubular epithelium of the kidneys and the lobular epithelium of the liver.

4. Nephritis of rabbits materially reduces tolerance for sulfanilamide, and the compound should be given in reduced dosage and with extra caution to human beings with defective kidney elimination.

5. The compound is rapidly absorbed from the subcutaneous tissues and gastrointestinal tract of rabbits. In one-half hour after oral administration considerable free sulfanilamide can be found in the blood, and maximum amounts of both free and conjugated sulfanilamide are found after two to three hours. Little or no conjugated sulfanilamide is present in the blood as early as one-half hour after oral administration.

6. Elimination is also quite rapid in the normal rabbit since only a negligible amount of free sulfanilamide is found in the blood after about thirty-two hours. In nephritic rabbits, however, elimination is materially delayed and conjugated sulfanilamide accumulates to a greater extent than free sulfanilamide with an increase of toxicity.

7. The maintenance of 10 to 12 mg. of free sulfanilamide per 100 c.c. of blood, believed to be required for the maximum of therapeutic effectiveness, requires in the rabbit the administration of almost maximum tolerated or toxic amounts of the compound.

8. Conjugation of sulfanilamide appears to occur in the blood and fixed tissues, but its therapeutic effectiveness is uncertain and unknown at the present time.

9. The intradermal inoculation of rabbits with virulent beta hemolytic streptococcus produces a severe local lesion without septicemia available for clinical observation and bacteriologic examination for evaluating the therapeutic effectiveness of chemical agents. The simultaneous intravenous injections of culture results in the production of septicemia and a large percentage of animals develop suppurative arthritis. These lesions are believed to be more useful than streptococcus infections of mice for chemotherapeutic investigations.

10. Sulfanilamide in the doses employed usually produced prompt and marked clinical regression of the local streptococcus skin lesions, but only irregularly and occasionally complete bactericidal effects. Small amounts of free sulfanilamide were found in these lesions, but not in the amounts regarded as required for sterilization of the blood.

11. About 83 per cent of untreated control rabbits developed arthritis, and 50 per cent developed this secondary infection under sulfanilamide treatment.

12. In all untreated control rabbits arthritis progressed and all died with positive blood and joint cultures. In a series treated with sulfanilamide the joint lesions continued to progress in 75 per cent, and 75 per cent succumbed with positive blood and joint cultures.

13. Sulfanilamide usually increased the phagocytosis of streptococci in the local skin lesions, and it appears that at least part of the therapeutic effectiveness of the compound consists in producing sufficient bacteriostasis and destruction of toxins to promote phagocytosis.

14. While sulfanilamide represents a marked advance in the chemotherapy of hemolytic streptococcus infections, especially of septicemia, there is urgent need for the production of compounds more effective in the disinfection or sterilization of the primary and secondary fixed tissue infections.

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LABORATORY METHODS

A RAPID AND SIMPLIFIED METHOD OF EXTRACTING URINARY ESTROGEN*

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THE biochemical estimation of estrogenic substance is admittedly an important step in the study of functional disorders of the female genital tract. The more general adoption of such hormone studies will depend primarily upon the rapidity and simplicity of the procedure. The improved apparatus described herein meets these requirements. The extraction of urinary estrogen is completed within three hours, and has given us results which compare favorably in every way to other methods.

Prior to 1932, two procedures were commonly used; namely, the chloroform method of Frank and Goldberger,[1] and the ethyl acetate method of Clarke and Kurzrok.[2] The latter method has been used in our laboratory for the past five years because it proved to be more easily assembled and required less attention. The duration in which the droplets of ethyl acetate were in contact with the urine was so short that it was necessary to continue the extraction for twenty-four to forty-eight hours to secure complete extraction of the estrogenic substance. In 1935, Smith and Smith[3] advocated a benzene method which they believed gave a more efficient estrogen assay than chloroform, olive oil, or ethyl acetate. The fire hazard with benzene requires the extraction to be conducted under extreme precautions, a serious handicap to its general use. These authors stressed the importance of subjecting the urine to hydrolysis in order to release any combined estrogen. It is very likely that this preliminary treatment of the urine accounts for the greater efficiency of the Smith and Smith procedure.

Estrogenic substance is excreted in a combined form to a considerable extent in pregnancy urine, and to a much less extent in the urine of nonpregnant mature females. In this combined form it is fat-insoluble and biologically inert.[4] By subjecting the urine to bacterial hydrolysis (permitting the urine to incubate for forty-eight hours or remain at room temperature for three days), or by boiling the urine with acid (acid hydrolysis), the combined estrogen is "freed" and made extractable by the fat solvent.

Our experience with acid hydrolysis of the urine preliminary to using the Clarke and Kurzrok set-up was very unsatisfactory because the ethyl acetate was broken down into acetic acid by the treated urine. This occurred in spite of cooling and neutralizing the urine before setting up the extraction.

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In seeking for another method, we combined the advantages of all the aforementioned procedures and developed the new apparatus shown in Fig. 1.

METHOD

The apparatus is assembled as in Fig. 1 with the exception of the condenser *D*. The Erlenmeyer flask is of 100 c.c. capacity and is connected to the arm *F* by a cork stopper.

Sufficient chloroform is placed into the extractor *B* to reach a level about 2 inches higher than the glass disk *C*.

Five hundred cubic centimeters of a measured twenty-four-hour collection of urine are acidified with 50 c.c. of concentrated hydrochloric acid, and the

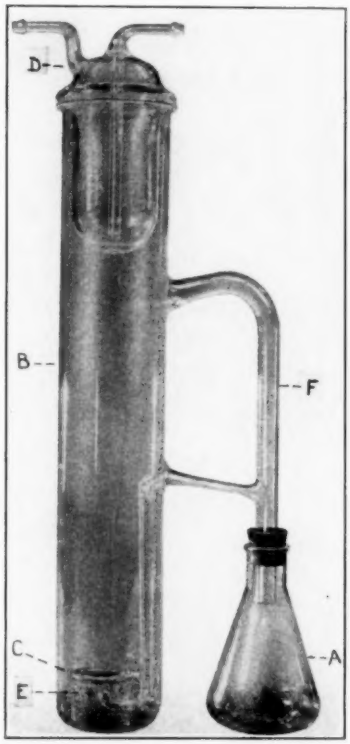

Fig. 1.

urine evaporated to one-third its volume by boiling. It is then cooled and poured into the extractor *B* through a long-stemmed funnel. The urine will form a layer above the chloroform.

Additional chloroform is slowly poured into the extractor *B* until the chloroform is seen to pass through tube *E* into flask *A*. Enough chloroform is added to allow about 25 c.c. of chloroform to collect in the flask.

The condenser *D* is set in place and the cold water turned on. Heat is then applied to the extractor and the flask.

When the chloroform in the extractor is brought to a boil, at first it rises partly into the layer of urine and slowly drops back again. When the tempera-

ture of the urine is elevated to that of the boiling chloroform, the chloroform will bubble vigorously through the layer of urine and vaporize into the column of air above where it is reliquefied by the condenser. At this stage an emulsion will form between the layer of chloroform and the urine. The flame must now be lowered to prevent the emulsion from entering the tube *E*. Once this point is reached the apparatus need not be watched any longer until the extraction is completed.

While the above is taking place, the chloroform in the flask is also being vigorously heated. The chloroform vapors from the flask pass through the arm *F* into the extractor and are condensed. The increased volume of chloroform in the extractor causes the chloroform at the bottom of the extractor to rise in tube *E* and spill over into the flask. This performance is a continuous one.

Within two hours all the chloroform-soluble substances of the urine have been completely extracted and transferred to the flask.

The flask is then disconnected. Five cubic centimeters of propylene glycol (olive oil or cotton seed oil may be used instead) are added to the chloroform extract, and the chloroform driven off by heating over a water bath until no odor of chloroform is detected. The remaining extract is used for assay purposes, 1 c.c. of the extract representing 100 c.c. of urine.

SUMMARY

A new apparatus with a high efficiency of extraction of urinary estrogen is described. The period of extraction is reduced to less than three hours. The method advocated can be readily adopted by any laboratory because of its simplicity and its safety.

The authors wish to express their appreciation to Dr. Joseph C. Ehrlich, Director of Laboratories, for his helpful assistance in the elaboration of the apparatus described herein. We are also indebted to the Empire Laboratory Supply Co., New York City, for their generous cooperation in the development of the apparatus.

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THE STABILITY OF KLINE ANTIGEN EMULSIONS*

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THE Kline precipitation test for syphilis is widely employed because of its simple technique, accuracy, and relatively low cost. One tenth milliliter of antigen will yield sufficient antigen emulsion for several hundred diagnostic tests. Since the antigen emulsion is considered as satisfactory for use for only forty-eight hours after preparation, there are many laboratories which will use only a small portion of the antigen emulsion within this time.

A lot of the diagnostic antigen emulsion was prepared on August 19, 1937, and was placed in an icebox by error. The emulsion was discovered several days later and was tested against known positive and negative sera; the results agreed perfectly with those which were obtained with freshly prepared antigen emulsions. This lot of emulsion was stored in an icebox and was checked at frequent intervals; reliable results were obtained for four months, after that, there was a progressive increase in sensitivity; and at the end of five months, it gave strongly positive reactions with negative sera. In the meantime, other lots of emulsion have been treated in the same way, and they have given reliable results for relatively long periods.

It would appear that Kline diagnostic antigen emulsions, when stored in an icebox, are relatively stable and may be used for at least several weeks. Naturally, the antigen emulsion should be tested against known positive and negative sera prior to use.

AN INEXPENSIVE PORTABLE ELECTRICAL THERMOMETER FOR THE HYPERPYREXIA CLINIC†

FRANCIS W. BISHOP, ROCHESTER, N. Y.

IN ROUTINE hyperpyrexia it is desirable to employ some type of continuously indicating thermometer. This is especially true in the case of prolonged fever bouts (ten to twenty-four hours). The use of such an instrument spares the patient a great deal of trauma and, on the whole, greatly facilitates the accurate administration of the fever. Of the electrical instruments for this purpose, either the thermocouple or the resistance thermometer may fulfill the requirements. The principal disadvantages of these devices have been the bulk, complexity, and cost. For the past six years, we have used both

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thermocouple and resistance thermometers for the rectal thermometry, but the instrument of our choice has been the resistance thermometer.

We have attempted to construct a suitable instrument at the lowest possible cost, and it is our purpose to describe a resistance thermometer unit which may be duplicated in most laboratories for $75.00. One of the principal difficulties was to find a galvanometer of the necessary accuracy and sensitivity

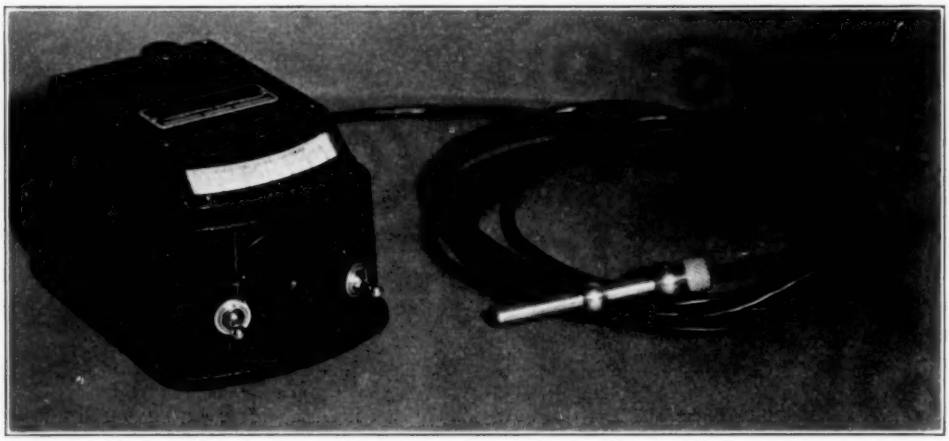

Fig. 1.—The thermometer is shown completely assembled. Adjustment shafts for R_5 and R_6 are in back of case. Note: External finding posts are standard with this galvanometer unless otherwise specified.

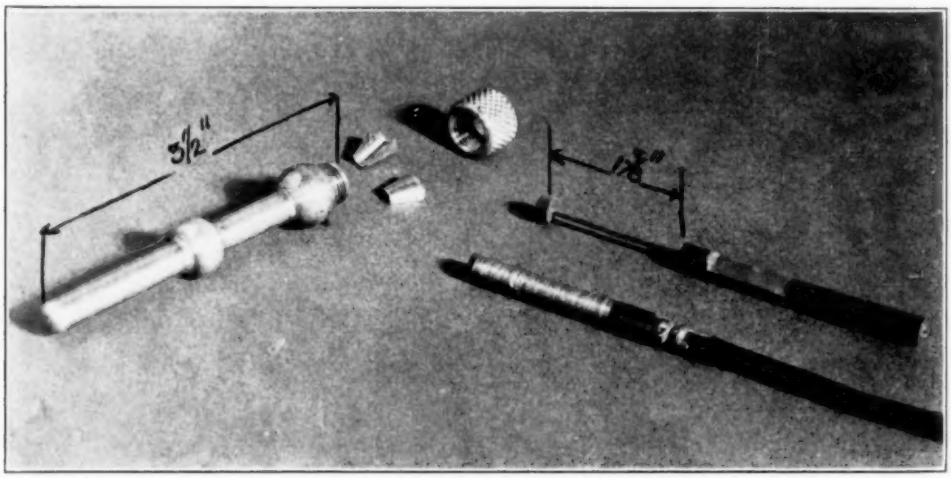

Fig. 2.—This figure shows the steps of assembly of temperature—responsive element and split cone-locking device to prevent cable from being pulled out of its housing. Winding is shellacked and housing filled with wax to reduce thermal lag.

which was not too expensive. This has been very well met in the G-M No. 2562-B pointer-type D'Arsonval galvanometer, costing $20.00 (Fig. 1). This instrument has a resistance of 300 ohms, a sensitivity of 0.25 microampere per mm. deflection, and a 60 mm. scale division, which is calibrated in degrees Centigrade from 36.0° to 42.0°. Each millimeter scale division thus equals

0.1° C. The galvanometer is of the taut suspension type, and is more rugged and less susceptible to damage than the ordinary jewel-bearing meter. They may be obtained in various sensitivities to suit the resistance thermometer used. The thermometer bulb itself consists of a coil of nickel having a resistance of about 600 ohms (569.21 at 36.0° C. to 609.05 at 42.0° C. in one case). The wire is wound on an insulating bobbin and is placed in a thin brass housing of the proper size and shape to be retained in the rectum (Fig. 2). This item can probably be purchased or constructed for $20.00 or less. In the bridge circuit in Fig. 3, resistances R_1 and R_2 are Leeds and Northrup manganin wire unmounted resistors, costing $1.50 each. Resistor R_3 is specially wound to

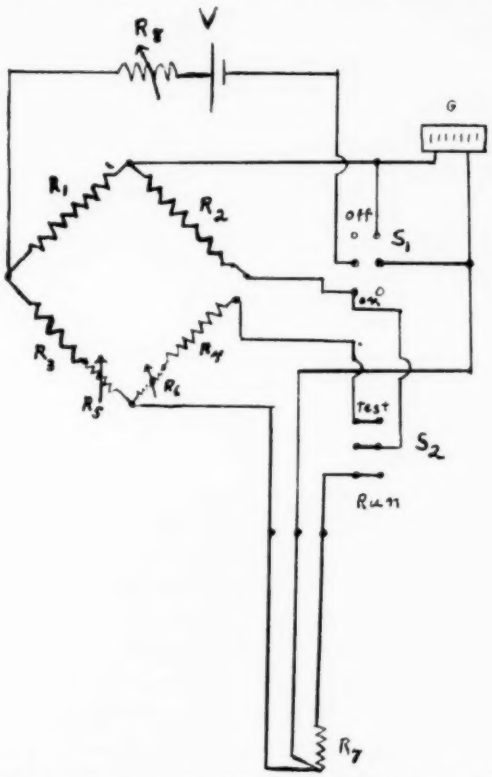

Fig. 3.—Complete wiring diagram of resistance thermometer. *V*, 1 No. 950 flashlight cell; *G*, No. 2562 G-M 300 ohm pointer type galvanometer; R_1, 500 ohm Leeds and Northrup No. 4584 unmounted resistor; R_2, 500 ohm Leeds and Northrup No. 4584 unmounted resistor; R_3, Leeds and Northrup No. 4584S unmounted resistor 2¼ ohms less than R_7 at 0, or 36.0° C; R_4, Leeds and Northrup No. 4584S unmounted resistor 2½ ohms less than R_7 at full scale, or 42.0° C.; R_5, 5 ohm wire wound radio control; R_6, 5 ohm wire wound radio control; R_7, about 100 ft. No. 40 nickel wire wound on suitable form—resistance between 500 and 600 ohms; R_8, 500 to 1000 ohm wire wound radio control; and S_1 and S_2, double pole, double throw radio toggle switches.

have a resistance of 2½ ohms less than the resistance of the thermometer at 36.0° C., while resistor R_4 is adjusted to 2½ ohms less than the resistance of the thermometer at 42.0° C.

Resistors R_5 and R_6 are ordinary 5 ohm wire wound variable radio rheostats. The shafts of these rheostats are slotted for adjustment with a screw driver, and once set are not disturbed. Variable rheostat R_8 is wire wound, has a resistance of 500 to 1,000 ohms, and is used to compensate for any

change in the battery voltage. Battery "V" consists of one No. 950 flashlight cell. The current drain is 2.5 Ma., which should provide some months of ordinary use. Flashlight cells are used in order to eliminate any external batteries or connections, and so to make the unit entirely portable and self-contained. There are two small double-pole, double-throw toggle switches. One of these is used to turn the unit off or on and when this switch is in the "off" position, the galvanometer is short-circuited. The second switch is used to connect either the thermometer or the test resistance R_4 to the bridge.

To calibrate the resistance thermometer, the bulb (R_7) is placed in a water bath at 36.0° C. The switch is placed in "run" position, and resistance R_5 is adjusted to bring the galvanometer to zero or 36.0° C. The temperature of the thermometer is raised to 42.0° C. and resistance R_8 is adjusted to bring the galvanometer reading to 42.0° C., or full scale. The switch is then thrown to "test" position, and resistance R_6 is changed until the galvanometer reads 42.0° C. Resistances R_5 and R_6 are not further changed, the galvanometer deflection being standardized at any time simply by throwing the switch to "test" position, and then, if necessary, by moving the control rheostat R_8 to bring the needle to 42.0° C. The switch is then thrown to "run" position, and the galvanometer deflection will indicate the temperature of the thermometer bulb if its temperature is between 36.0° C. and 42.0° C.

We attempted to use good quality radio resistors throughout, but these were so affected by ambient temperature and humidity changes as to be useless. Resistors R_5 and R_6, however, comprise such a small proportion of the total resistance as to be negligible in their variations. The other fixed resistors, R_1, R_2, R_3, and R_4, should be high quality manganin coils. The resistances of the arms of the bridge and of the thermometer coil should be fairly high in order to keep the drain on the battery low. The temperature responsive element is connected to the bridge by a small flexible three-wire cable. This connection shown in Fig. 3, eliminates the effects of changes in resistance of the leads with temperature. The unit may be calibrated in degrees Fahrenheit or Centigrade, and special scales are obtainable from the manufacturer of the galvanometer. If temperature control is desired by means of the above-described apparatus, the galvanometer may be substituted by the G-M Super-Sensitive D'Arsonval galvanometer relay, with no loss of sensitivity. This instrument includes the same galvanometer as before, but with a synchronous motor driven contacting device, which may be used with a secondary relay for the automatic control of the patient's temperature.

This unit, in common with other electrical thermometers, will not function properly where high frequency fields are employed to produce the fever.

A simple electrical thermometer has been described, the cost of which brings it within the reach of any hyperpyrexia clinic.

THE COMPLEMENT FIXATION TEST IN CHANCROIDAL INFECTION*

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ELSEWHERE[1-3] we have described the results of the skin test, utilizing a bacillary antigen, in the differentiation of chancroidal infection. From a clinical standpoint this test has proved very satisfactory. The one drawback, if such it may be called, is due to the fact that the test having to be read forty-eight hours after injection of antigen, necessitates a revisit to the clinic on the part of the patient. Sometimes this is a hardship, and sometimes the patient fails to return, with the result in such cases that the test must be repeated.

Positive skin reactions in chancroidal infection are undoubtedly antigen-antibody combinations, and so it was felt that some in vitro serologic test might be of equal value, and thereby eliminate the revisit of the patient. Owing to the methods we found to be essential in the cultivation of *H. ducreyi,* together with the peculiarities in the growth of this organism, an agglutination test was out of the question. The complement fixation test suggested itself as an alternative.

METHODS†

The complement fixation test adopted utilized two units of antigen, one and one-half units of complement, 0.1 c.c. of test serum, rabbit antisheep hemolytic system, and adjusted to a total volume of 2.5 c.c. with saline. Fixation employed the 37° C. water bath throughout: one hour preliminary, and one-half hour final. Since the antigen was found to be the crux of the whole situation, its preparation will be described.

The strains of *H. ducreyi* which we had isolated grew very poorly on the surface, and it was this observation which led us to adopt the method of cultivation and preparation of skin test antigen previously described.[1] Essentially, this consisted of growing the organism in defibrinated human blood added onto the surface of infusion agar slants, reducing the oxygen tension by gently heating the side of the tube, and then tightly stoppering it. After three days' incubation at 37° C., the blood and growth were removed to sterile distilled water and centrifugated at high speed. The sediment was washed until the supernatant was free of hemoglobin—usually twice—and to the final sediment was added sterile physiologic saline to the extent of 10 c.c. per agar slant. The antigen was heated for forty minutes at 60° C., and 1:10,000 merthiolate

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†Since the completion of these studies, our attention has been called to a publication by Ravaut, Rivalier, and Cachera, Presse méd. **40**: 409, 1932. Nine cases of chancroid were studied, and positive complement fixation tests were obtained in all. Details of methods and technique were not given. It was stated, however, that dmelcos vaccine was used as the antigen—a preparation not commercially available to the American market. We have reported comparative skin tests using dmelcos vaccine and our own preparations, and the results were always similar.[2]

TABLE I

COMPLEMENT FIXATION TESTS

CASE	ANTIGEN (HUMAN BLOOD GROWTH)	ANTIGEN (RABBIT BLOOD GROWTH)	CHANCROID SKIN TEST	FREI TEST	REMARKS
1	2- 4 negative 3-11 negative 3-25 negative 5- 6 negative	4- 1 4 plus 4- 9 4 plus 4-15 4 plus 4-23 4 plus 5- 6 4 plus	positive	negative	Granuloma inguinale Donovan's bodies present Fusospirochetosis
2	2- 4 negative 3-11 negative	----------	positive	negative	Clinically chancroid Multiple lesions 1-14
3	3-11 negative	----------	positive	positive	Chancroid; ulceration for 6 months
4	3-11 negative	4-23 4 plus 5- 6 4 plus	positive	negative	Chancroid; ulcers a few weeks
5	3-11 negative 3-25 negative	----------	positive	negative	Clinically chancroid; ulcer at fourchette 2 weeks
6	3-25 negative	----------	positive	positive	Clinically chancroid; bubo present; had bubo 1 year ago also
7	3-25 negative	----------	negative	positive	W.R. became positive after 5 weeks; hard bubo, probably syphilitic
8	3-25 negative	4- 9 4 plus 4-15 4 plus 4-23 4 plus	negative	positive	Lymphogranuloma venereum Rectal stricture
9	3-25 negative	----------	doubtful	positive	Probably lymphogranuloma venereum. Ulcer on fourchette for 2 weeks
10	3-25 negative	4- 1 3 plus 4- 9 4 plus 4-15 4 plus 4-23 4 plus	positive	positive	Lymphogranuloma venereum Perianal abscess
11	3-25 negative	4- 1 4 plus 4- 9 4 plus 4-23 4 plus	negative	positive	Lymphogranuloma venereum Elephantiasis of vulva
13	3-25 negative	----------	negative	positive	Lymphogranuloma venereum
14	----------	4- 1 negative 4- 9 3 plus 4-15 2 plus 4-23 1 plus	negative	positive	Lymphogranuloma venereum (rectal)
15	----------	4- 1 2 plus 4- 9 4 plus	positive	weak	Probably chancroid
16	----------	4- 1 3 plus 4- 9 4 plus 4-23 4 plus	weak	positive	Probably lymphogranuloma venereum. Multiple small ulcers on cervix
17	----------	4- 1 4 plus 4- 9 4 plus	negative	negative	Acute G.C.
18	----------	4- 1 1 plus 4- 9 4 plus	negative	positive	Lymphogranuloma venereum. Bilateral buboes. No primaries

TABLE I—CONT'D

CASE	ANTIGEN (HUMAN BLOOD GROWTH)	ANTIGEN (RABBIT BLOOD GROWTH)	CHANCROID SKIN TEST	FREI TEST	REMARKS
19	----------	4- 1 negative 4- 9 4 plus 4-23 3 plus	negative	positive	Clinically indefinite
20	----------	4- 1 4 plus 4- 9 4 plus	positive	positive	Wassermann 4 plus. Clinically mixture of syphilis, lymphogranuloma venereum, and chancroid
21	----------	4- 1 negative 4- 9 negative	positive	weak	Clinically chancroid
22	----------	4- 1 negative	positive	negative	Clinically chancroid Multiple penile sores
23	----------	4- 1 3 plus	positive	negative	Clinically chancroid, 7 months' duration
24	----------	4-15 4 plus 4-23 4 plus	positive	negative	Clinically granuloma inguinale. Donovan's bodies present, several years' duration
25	----------	4-15 4 plus 4-23 1 plus	weak	positive	Developed bilateral buboes. W. R. negative then became positive; probably syphilitic
26	----------	4-23 1 plus	positive	negative	Clinically chancroid
27	----------	4-23 4 plus	weak	positive	Clinically granuloma inguinale. Donovan's bodies present
28	----------	4-23 3 plus	negative	positive	Small lesions on cervix Fusospirochetosis
29	----------	4-23 4 plus	positive	negative	Chancroid. Vulval lesion 3-10; bubo 3-15
31	----------	4-23 1 plus	positive	negative	Chancroid. Multiple lesions of vulva; first appeared 4 months ago
32	5- 6 negative	5- 6 4 plus	positive	negative	Chancroid. Lesions with bubo
33	5- 6 negative	5- 6 3 plus	positive	?	Chancroid. Penile lesion with bubo, 1 month
34	5- 6 negative	5- 6 4 plus	positive	positive	Chancroid. Ducrey's bacillus isolated. Labial ulcer for 9-12 months
35	5- 6 negative	5- 6 1 plus	positive	positive	Chancroid. Ducrey's bacillus isolated. Lesion with bubo for 3 weeks
36	5- 6 negative	5- 6 4 plus	negative	negative	Suspicious Ducrey's bacillus in smears. Two labial ulcers for 2 months
37	5- 6 negative	5- 6 4 plus	negative	positive	Lesion on vulva which healed quickly; probably lymphogranuloma venereum

added as a preservative. The erythrocytic stroma gives this antigen a "murky" appearance, but nevertheless, such preparations have yielded entirely satisfactory results in the skin test during the past two years of our observation.

The antigens for use in the complement fixation tests were prepared as above, using the same strains, but with the variation that 5 c.c. of saline per slant were substituted for 10 c.c. These antigens were titrated against hyperimmune rabbit serum, and although fixation was obtained, it was found that no more than two antigenic units could be satisfactorily used because the murkiness interfered with the reading of hemolysis. The rabbits were immunized with killed cultures, grown in rabbit blood.

In the preliminary tests on sera from chancroidal patients, the results were entirely negative. To corroborate this, as many as possible of our clinic chancroidal patients were rounded up. Tests on their sera were also negative. They were also negative in patients who on the basis of a positive skin test had had chancroid in the past (Table I). Later in the course of the studies, an antigen made from growth in *rabbit* blood was tested. Positive fixations were obtained.

TABLE II

COMPLEMENT FIXATION TESTS USING HEMOLYZED, WASHED RED BLOOD CELL RESIDUE AS ANTIGENS

CASE*	RABBIT CELLS	HUMAN CELLS	REMARKS
1	4 plus	negative	Granuloma inguinale
4	4 plus	negative	Chancroid
24	4 plus	negative	Granuloma inguinale (old chancroid)
27	4 plus	negative	Granuloma inguinale
32	4 plus	negative	Chancroid
33	4 plus	negative	Chancroid
34	4 plus	negative	Chancroid
35	2-3 plus	negative	Chancroid
36	3 plus	negative	Chancroid
37	4 plus	negative	Probably lymphogranuloma venereum

*Numbers correspond to those in Table I.

An additional number of known sera were tested, and as controls some 82 routine Wassermann sera from the health department. All of the sera from the chancroidal patients gave complete fixation as did 34 of the controls. The clinical histories of the latter were unknown, but from our two-year studies on chancroidal infection, it was felt that this apparent high incidence was altogether out of line. Still, the discrepancy in the two antigens could not be accounted for. Six sera from known chancroidal cases were then tested simultaneously with the two antigens: that made from human blood gave negative fixations, while that made from rabbit blood yielded complete fixation in all but two instances—a one plus and a three plus. A few tests using the rabbit blood antigen were made on ward patients having negative skin reactions and in whom there was no evidence past or present of chancroidal infection. Complete fixations were obtained. It was evident, therefore, that something inherent in the antigens made from growth in rabbit blood was responsible for the positive fixations observed. To substantiate this opinion, "antigens" were prepared using just the washed sediment, appropriately diluted, from laked human and rabbit bloods from amounts of blood that were customarily added to the agar slants when cultures

were grown. These were tested simultaneously with sera from six known chancroidal cases, one old (?) chancroid and three granuloma inguinale (Table II). The former "antigen" gave negative reactions, whereas the latter gave all positive. The rabbit "antigen," however, yielded negative results when tested against homologous serum. It is evident, therefore, that *all* the fixations obtained were undoubtedly due to a nonspecific fixation between residue rabbit blood cells and human sera.

SUMMARY AND CONCLUSIONS

1. Antigens which were satisfactory in the skin test for chancroidal infection and prepared from growth in human blood, gave negative results when used in the complement fixation test.

2. Antigens prepared from rabbit blood growth yielded false positive reactions in the complement fixation test, and were due apparently to some inherent quality of the rabbit blood cells.

3. With the antigens available, the complement fixation test was an unsatisfactory procedure in the diagnosis of chancroidal infection.

The authors wish to thank Dr. Anna D. Dulaney, Department of Bacteriology, University of Tennessee Medical School, for supplying sera and data on several chancroidal cases.

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A MULTIPLE RING MAKER FOR THE KLINE TEST*

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NUMEROUS devices have been described to facilitate the performance of the Kline test.[1, 2] A rigid brass multiple ring maker has been found to reduce the time required to make the paraffin rings for the Kline diagnostic and exclusion tests.

Cut twelve one-inch lengths of brass tubing 16 mm. (5/8 inch) outside diameter and about 1.2 mm. wall thickness. The tubing should be cut squarely, preferably by a mechanical hack saw. These short brass tubes are soldered to a heavy brass plate (3/32 to 1/8 inch thick). This brass plate should be of the same size as the glass slides used in the test. The position in which the brass tubes are soldered to the brass plate should correspond to the desired position of the paraffin rings on the Kline slide, usually three rows of

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four tubes. Space is saved if the rows are somewhat staggered. There must be a space between tubes, so that an excessive amount of paraffin is not held at the point of contact between tubes.

After soldering in place on the brass plate, the free surface of the bank of tubes is dressed with a file till the ends of the tubes are in the same plane. Further grinding to a plane surface is accomplished by rubbing on emery paper which has been glued to plate glass to prevent buckling. Final grinding is accomplished by rubbing the surface of the bank of tubes on plate glass sprinkled with emery powder, being careful not to tip the instrument at any time during the grinding to avoid grinding more off of the edges than the center. When completed, the free ends of the brass tubes should all make contact with a plane surface throughout their entire circumference.

To furnish a vent, a small hole is drilled through the plate in the center of each tube. A heat-resisting handle, such as a bakelite radiator valve handle, is fastened by a screw to the top of the plate to complete the instrument, which when finished resembles a rubber stamp in appearance (Fig. 1).

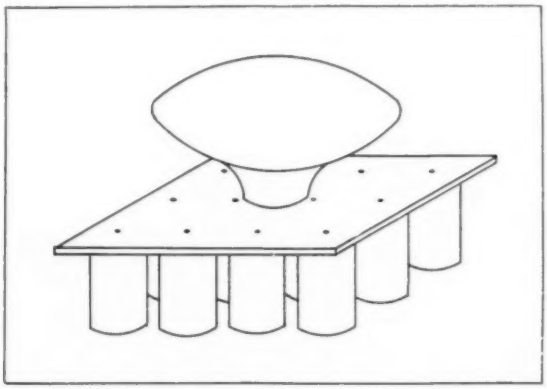

Fig. 1.

It is used in a similar fashion to a rubber stamp by dipping the instrument into melted paraffin (about 80° C.), allowing the stamp to become warm, shaking off the excess paraffin, and then transferring to the Kline slide.

The resulting height and width of the paraffin ring is regulated by adjusting the temperature of the paraffin, the temperature of the brass stamp itself, the depth to which the stamp is immersed in the paraffin bath, the temperature of the glass slide to which the transfer is made, and the time intervening between removal of the stamp from the paraffin bath and its application to the slide. Practice will enable the user to determine the most satisfactory conditions for use.

This ring maker possesses the advantage of perfect rigidity. If the surface of the ends of the brass tube becomes marred, it is easily resurfaced by repeating the grinding with emery as above described.

It has also been found useful to control the radius of rotation of the tray containing the Kline slides by the simple method of fitting a small short peg

into the bottom of the tray. This peg fits into a large hole in a board placed on the table or, if desired, into a hole in the laboratory table itself. The radius of the peg, when subtracted from the radius of the hole, will give the radius of rotation. In operation the small peg is made to travel around the circumference of the large hole, thus limiting the radius of rotation. This prevents the otherwise frequent spilling of the contents of the rings.

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TRIAXIAL CORRELATION OF HEMATOLOGIC INDICES: ITS SIGNIFICANCE IN CLASSIFICATION AND TREATMENT OF ANEMIAS*

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THE importance of hematologic indices in the diagnosis and treatment of anemias is now so well established in clinical practice that even a cursory discussion of the subject may seem superfluous, yet the definite relationship existing between the color, volume, and saturation indices is but vaguely appreciated by the average clinician. The exposition of fundamental principles involved in the application and interpretation of index numbers as given in most hematologic textbooks is unavoidably vitiated by pages of awkward equations that are hardly inviting to the eyes of busy practitioners. To offset this shortcoming, a few authors, particularly Osgood (1927, 1935), have constructed tables and charts whereby the indicial figures may be obtained at a glance without resorting to tedious though simple arithmetic. That these three indices are, as a matter of fact, mutually interdependent and, therefore, amenable to a simple mathematical correlation by means of a suitable graphic method has never been pointed out even by hematologists. The graphic chart here presented is an attempt to offer a simple, accurate, and practical correlation of the indices, with special reference to classification and treatment of anemias.

Each of the three major hematologic indices of diagnostic importance, the color index (Hayem, 1878), the volume index (Capps, 1903), and the saturation index (Haden, 1923), is expressed in relative numbers, the unit 1.0 representing the value for a normal blood picture. Upon mathematical manipulation of indicial values under both normal and pathologic conditions, a complete and rather constant relationship is found to exist between them.

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This interrelationship is clearly demonstrable when the indicial values are expressed in their logarithmic equivalents.
Thus, since

$$z = \frac{x}{y},$$

in which x represents the value for the color index, y the volume index, and z the saturation index,

$$\log z = \log x - \log y,$$

therefore,

$$\log x - \log y - \log z = 0.$$

This is an equation of the general form:

$$ax + by + cz = K,$$

in which the three variables are mutually related and can be expressed on the triaxial chart, as Hastings and his co-workers (1931, 1934) have done in

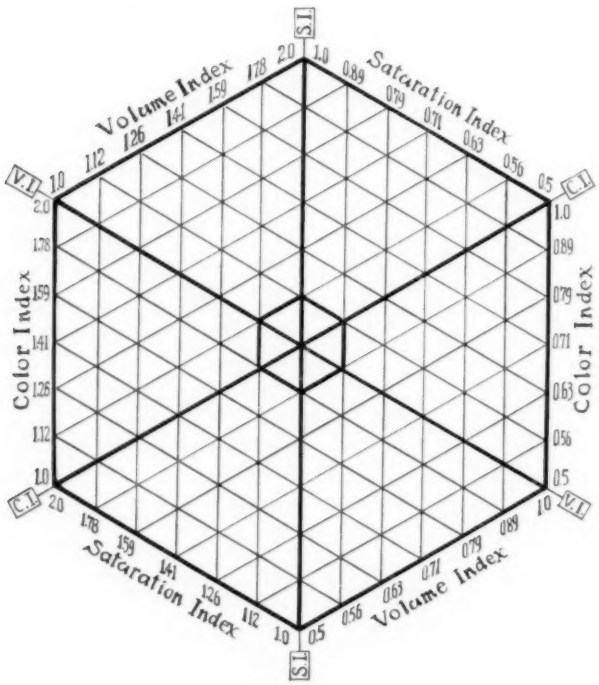

Fig. 1.

their studies on the acid-base balance of the blood under normal and experimental conditions. Hematologic data as converted into index numbers are likewise suitable for plotting on triaxial coordinate papers.

CONSTRUCTION OF THE TRIAXIAL CHART

The triaxial chart (Fig. 1) here presented is so constructed, following the general procedure as employed by Hastings and Steinhaus (1931), that the three axial lines connecting the six corners of the hexagon represent the ideal normal values for color index, volume index, and saturation index, respectively, and all meeting at a point in the geometric center. This point,

therefore, represents the uniform value of 1.0 for all three indices, from which deviations of ± 0.1 are customarily regarded as being within the normal limits. This range of normal variations is represented by a smaller hexagon enclosed by heavy lines within the center of the chart (see Fig. 2). The axis connecting II and VIII o'clock represents the indicial value of 1.0 for the color index; and the parallel lines, both above and below this axis, denote the varying degrees of graduation as indicated on the left and right margins of the chart, respectively. Likewise, the axial line, passing in the direction of IV and X o'clock, indicates the value of 1.0 for the volume index, with similarly varying graduations both above and below in parallel lines. The normal value of 1.0 for the saturation index runs in the vertical direction of VI and XII o'clock, with its sets of parallel lines on either side of the main axis.

The marginal figures of the hexagonal chart represent three sets of relative numbers corresponding to the three indices, each set being expressed in exactly comparable values, ranging from 0.5 to 2.0, with 1.0 at the axial

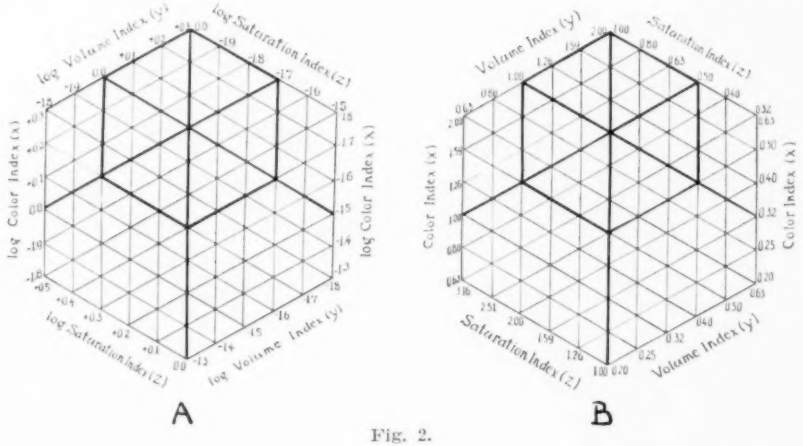

Fig. 2.

positions. The logarithmic values are placed at regular and evenly spaced intervals on the chart (Fig. 2 A), and the indicial numbers correspond each to its own logarithmic equivalent (Fig. 2 B). The actual portion of the coordinate field utilized for the present chart is demarcated by heavy lines at the top of the original chart.

Thus, the triaxial chart as applied to indicial numbers is a strictly accurate mathematical expression of the actual interrelationship existing between the three indices. The main advantage of the chart is its convenience in locating the indicial position of a given blood picture, with special reference to its hematologic classification, and in recording the progress of the anemia under therapeutic management, as well as in determining its saturation index. These features distinguish the triaxial coordinate chart here presented from the simple calculation chart of Osgood (1927, 1935).

In the practical use of the chart for hematologic diagnosis, the color and volume indices are first calculated from actual determination of red cell count per cubic millimeter of blood, of the amount of hemoglobin in grams per

100 c.c., and of the volume of packed cells in percentage of the whole blood.* Since the saturation index is a quotient obtained by dividing the color index by the volume index, its graphic position can be automatically located on the chart once the color and volume indices are known. This obviates the necessity of calculating the saturation index separately.

NORMAL AND ARBITRARY STANDARDS

The calculation of hematologic indices requires the use of certain standards which may be either actual figures representing average values obtained from observations on normal individuals, or some simple round figures arbitrarily selected for convenience in calculation. Some authors (Osgood, Haskins, and Trotman, 1932) justly emphasize the fact that the standards with which any pathologic blood is to be compared must be actual average values

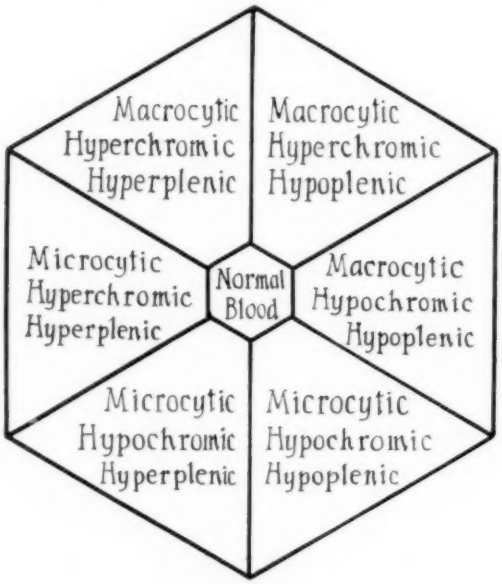

Fig. 3.

obtained from normal individuals of the patient's own age and sex group. This is entirely correct, especially if the comparison is to be made directly in absolute numbers. Such a set of average figures, which at least in certain age groups are available, may be designated as normal standards. It goes without saying that such standards must be derived from the most extensive observations on very large groups of normal individuals.

As a matter of fact, however, in the calculation of hematologic indices actual average figures of normal values are not absolutely necessary, inasmuch as the indicial numbers are always but relative in nature. Any round numbers which are fairly representative of the normal average values and at the same time simple enough for ready calculations can be used for this purpose

*In working with infants and small animals, in which capillary blood is more readily obtainable than is venous blood, the use of a recently devised microhemopipette (Kato) requiring only 0.05 c.c. of oxalated blood is recommended for the determination of cell volume (J. LAB. & CLIN. MED. 23: 980, 1938). If venous blood is available, other types of hematocrit, using larger amounts of blood, are satisfactory. In either case the proportion of oxalate to the blood should be 1:500 (or 1 mg. per 0.5 c.c.).

with complete satisfaction. These standards, called arbitrary standards, may thus be regarded as common factors which, when consistently used throughout all calculations, will yield strictly comparable indices. It is at once clear then that the use of simple arbitrary standards as common factors in indicial equations is preferable to employment of the more complex and consequently more troublesome sets of actual normal standards. The following set of figures, uniformly employed in all calculations, are customarily regarded as arbitrary standards, each representing the equivalent of 100 per cent as normal:

Erythrocytes (millions per c.mm.)	5.00	(Normal range: 4.50-5.50)
Hemoglobin (gm. per 100 c.c.)	15.0	(Normal range: 14.0-16.0)
Packed cell volume (per 100 c.c.)	45.0	(Normal range: 40.0-48.0)

The above standards are round figures which maintain a rather simple relation to each other, so that the calculation of indices is considerably simplified, as shown in the following paragraph.

CALCULATION OF INDICES

Using the set of arbitrary standards as recommended in the preceding paragraph, the calculation of hematologic indices may be obtained by means of the following equations:

$$(1)\ \text{Color Index} = \frac{\text{Hemoglobin (grams per cent)}}{\text{Erythrocytes} \times 3}$$

in which the factor 3 has been derived from the ratio that the figure 15.0 (arbitrary standard for hemoglobin) maintains to the figure 5.00 (arbitrary standard for erythrocytes).

$$(2)\ \text{Volume Index} = \frac{\text{Packed cell volume (per cent)}}{\text{Erythrocytes} \times 9}$$

where the factor 9 has been obtained from the ratio that the figure 45.0 (arbitrary standard for packed cell volume) maintains to the figure 5.00 (arbitrary standard for erythrocytes).

The corpuscular values of Wintrobe (1932, 1933) may also be converted into standard index numbers by use of the following equations:

$$(1)\ \text{Color Index} = \frac{\text{Corpuscular hemoglobin (C. H.)}}{30}$$

in which the denominator 30 is the arbitrary standard for mean corpuscular hemoglobin in average normal individuals, as expressed in micromicrograms.

$$(2)\ \text{Volume Index} = \frac{\text{Corpuscular volume (C. V.)}}{90}$$

where the denominator 90 is the arbitrary standard for normal mean corpuscular volume expressed in cubic microns.

$$(3)\ \text{Saturation Index} = \frac{\text{Corpuscular hemoglobin} \times 3}{\text{Corpuscular volume}}$$

in which the factor 3 represents the ratio that the figure 30 (arbitrary standard for normal mean corpuscular hemoglobin) maintains to the figure 90 (arbitrary standard for normal mean corpuscular volume).

CLASSIFICATION OF ANEMIAS BY THE TRIAXIAL CHART

The triaxial chart here presented may also be used for the purpose of accurately placing various types of anemias according to their indicial characteristics. The three axial lines serve as precise boundaries separating the six groups (Fig. 2). In the geometric center of the chart is a small hexagon which represents the limits of variations in normal blood, while the peripheral triangles classify the anemias with all the possible combinations of indicial characteristics. The terms macrocytic and microcytic customarily refer to the dimensional qualities of erythrocytes; hence macrocytic anemias have relatively higher volume index than the microcytic. Hyper- and hypochromic anemias are distinguishable primarily on the basis of color index values. The terms hyper- and hypoplenic, used for the first time as far as the writer is aware, refer to the degree of saturation of erythrocytes with hemoglobin as determined by the saturation index. Incidentally, as noted by Capps (1903), supersaturation of erythrocytes with the pigment very seldom, if ever, occurs. A low saturation index usually suggests an anemia due to chronic blood loss (Osgood, 1926).

The classification of anemias here evolved by means of the triaxial chart offers a definite aid in determining rational and specific therapy for each group. It is now known that macrocytic types of anemia respond favorably to liver and liver substitutes, while iron is the specific for the microcytic types. It is also clear that the anemia characterized by a combination of macrocytosis and hypochromia requires both iron and liver. Anemias with saturation index above 1.1, though extremely rare, respond poorly to any type of treatment, since this group postulates a highly abnormal developmental anomaly in erythropoiesis. It is quite conceivable, however, that it is this latter group of anemias which requires such therapeutic adjuncts as vitamins, iron catalysts, and other marrow stimulants, as well as additional substances directly or indirectly concerned in the formation of erythrocytes and hemoglobin.

The triaxial chart is, furthermore, well adapted for recording the course of progress under treatment. As a rule, the more severe the degree of anemia the more peripheral is the position of the blood picture on the chart. Under specific and effective therapy the indicial position of the blood will move toward the center, thereby indicating its return to the normal.

SUMMARY

A triaxial chart correlating the color index, volume index, and saturation index, together with simplified equations for calculation of these indices, is presented. An accurate hematologic classification of all types of anemia, so essential a foundation for rational therapy, is made possible by this chart. It also affords a simple and convenient method for recording the changes in the index values as influenced by specific treatment.

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PHOTOELECTRIC COLORIMETRY*

I. Determination of Blood Iron and Hemoglobin

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INTRODUCTION

THE inaccuracy of the eye in matching the intensity of colors is only too well known. This difficulty is not so marked in the blue-purple end of the color scale, but is quite pronounced in the red-yellow part. Also, the human eye is subject to the many variations of any organ of the body, the variation being even more accentuated with different individuals. Consequently, eye colorimetry, even in the blue end of the color scale, is far from being all that might be desired, and in the red part is often entirely unsatisfactory.

The photoelectric cell, however (which is essentially an electric eye), does not measure the intensity of colors as such, but measures only the degree of transmission of light; therefore, it reacts similarly to all colors even though it is somewhat more sensitive to the blue and purple shades. It is not subject to the variations of the human eye, having an accuracy characteristic of

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electrical instruments in general. Its introduction into the field of colorimetry has, therefore, been an important step, and augurs an era of more accurate chemical analysis in biological work.

In general there are two types of photoelectric colorimeters: those having one and those having two photoelectric cells. The one-cell instruments consist essentially of a photoelectric cell, a source of light of constant intensity (requiring a storage battery for its production), and a cup of standard size for interposing the solution to be tested between the light and the cell. Readings are taken with a set of standard solutions, and a curve plotted; the concentration of the unknown is then obtained by the location of its reading on the prepared graph. The "readings" may simply be the output of the photoelectric cell in amperes with different intensities of light; they may consist of the variation in the amount of light (by a calibrated shutter) or the electrical resistance necessary to keep the output of the cell constant with different color intensities. Such instruments are described by Kesten and Zucker,[1] Sheard and Sanford,[2-4] Ellis,[5] Evelyn,[6] Lebowich,[7] and others. Most of these workers employ the use of radio vacuum tubes and accessory batteries, or the use of galvanometers and other electrical material. The use of filters is advocated by some workers to "blot out" the parts of the spectrum not occupied by the color being measured.

The chief advantages of single-cell instruments are their simplicity in construction, their relatively low cost, and their ease of adaptability to the use of filters. Their disadvantages lie in the need for a constant source of light (furnished only by a battery), and the fact that fatigue of the cell and variations due to temperature changes, directly (and measurably) affect the colorimeter readings.

The two-cell instruments consist essentially of two photoelectric cells, a source of light (falling simultaneously upon both cells), a standard cup for interposing colored solutions, and some arrangement for measuring the changes in the system produced by different intensities of colors. The advantages of this type of apparatus are: first, the source of light need not be absolutely constant (eliminating the storage battery) since variations in the light affect both cells similarly; and second, the readings are taken by balancing the cells against each other by the use of a sensitive galvanometer (null-point method). The null-point method largely eliminates the errors arising from an inconstant light source, temperature changes, and cell fatigue. The instruments can also be adapted to a wider variety of uses, and the readings can be duplicated with different instruments.

Such instruments have been described by Exton[8-10] and Goudsmit and Summerson.[11] In Exton's instruments the two cells are connected to a galvanometer by suitable resistances, and the readings made as the amount of light (determined by the use of a calibrated shutter) necessary to keep the galvanometer reading constant when colored solutions are placed in the cup. The values for known solutions are plotted on a graph against the concentration, and the curve used to make the unknown determinations. In Exton's last instrument[10] the cells are mounted at 180° angles to each other, and the

light source is capable of being moved from one side to the other to produce a null-point reading on the galvanometer. This allows the direct comparison of standards and unknowns. Goudsmit and Summerson's instrument[11] is arranged like an ordinary colorimeter, with the eye being replaced by two photoelectric cells connected by suitable circuits to a galvanometer. The readings are taken by adjusting the depth of the colored solution in each cup until the galvanometer is at 0, indicating that the conduction of light (intensity of color) through each column of liquid is the same.

Although the null-point instruments are more desirable than the one-cell type, the initial cost is larger, and they present more difficulties to the use of filters. Our investigation of photoelectric colorimeters led us, therefore,

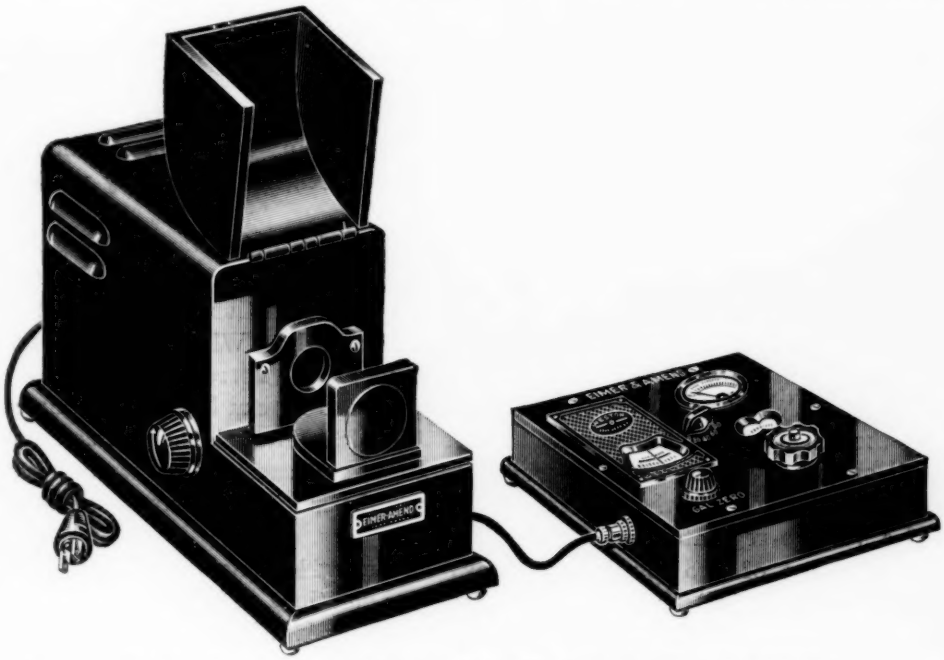

Fig. 1.—Complete photoelectric colorimeter (without voltage regulator).

to purchase an instrument made by the Eimer and Amend Co. of New York* (see Fig. 1). This apparatus is composed of two photoelectric cells using the same light source, the currents from the cells being balanced by a potentiometer (instead of adjusting the depth of the solution[11] or by a shutter arrangement[8-10]). The most important feature is that the first cell is circular in shape and surrounds the beam of light going to the second cell. In this way *one filter* can be interposed between the light source and both cells, rendering the use of filters a very simple matter. Readings are made directly as per cent conduction of light, by means of a suitable potentiometer (Weston). The cells are selenium on iron; they are quite stable and emit two or three

*The circuit is simple and the entire apparatus (except the potentiometer) can be easily constructed by anyone wishing to go to such trouble. The instrument made by Eimer and Amend has, however, proved quite satisfactory, and they have generously provided us with much of the information about the instrument that is given in this report.

times the amount of current given by most of the cells in current use. The instrument is also arranged for nephelometry by an adjustment of the second cell. The actual circuit is not given, but it consists essentially of two photoelectric cells connected to a galvanometer by a modified wheatstone bridge arrangement, the variable resistance being calibrated to read per cent conduction of light.

In the operation of the apparatus, the instrument is first allowed to "heat up" for fifteen to twenty minutes (for accurate work) in order to allow the output of the cells to become constant. The output of the first cell is then adjusted to some definite value, and the two cells balanced with water (or a blank of the reagents) in the interposed cup. Then the cup is filled with the unknown solution, and the per cent of conduction observed by again balancing the colorimeter. A graph is first plotted with solutions of known concentrations, and the concentration of the unknowns taken directly from the curve.

Suitable filters abet the determinations of most red, yellow, and green colors. We have used Wratten filters exclusively, and have had occasion to use only numbers 63, 47, and 29. These filters have not shown any measurable change with one year of use.

The use of a voltage regulator is desirable if the line voltage is irregular, especially when filters are employed. The location of the cells at different distances from the source of light causes a slight variation in the output of the cells, with variations in the intensity of the light. When filters are not used, the intensity of the light (as measured by the current output of the circular cell, and whose output *must* be kept constant) can be easily regulated by the rheostat on the instrument itself. If a filter is used, the photoelectric cell output is too small to be read accurately, and accurate values are best obtained by keeping the voltage coming into the machine constant by means of a voltage regulator.

The use of two solution cups may be desirable: one for the blank of reagents (or water) and one for the unknown solutions. However, these cups must be kept extremely clean (soap and water), as invisible traces of pigment dried on the surface of the cups affect the colorimeter readings measurably. This precaution may be neglected if only one cup is used for all solutions.

EXPERIMENTAL

In this work we used the photoelectric colorimeter described above. The slot for the use of filters had to be slightly enlarged to allow the use of Wratten filters. A voltage regulator was found essential as our current supply was quite inconstant. Filters were used to "spread" the conductivity readings over more of the scale, and a number 47 Wratten filter (gelatin) was found most suitable for both blood iron and hemoglobin curves.

Hemoglobin was determined by estimating blood iron, using the method and curve below (Fig. 2), and the hemoglobin values plotted against the conduction figures obtained from water dilutions (1:400) of whole blood (same sample). The curve (Fig. 3) was then drawn through the points plotted. The actual procedures in making the determinations are given below.

Method for Blood Iron.—Principle. This is the method of Wong[12] adapted to the photoelectric colorimeter. The iron in the blood is liberated with concentrated sulfuric acid, oxidized to the ferric state with potassium persulfate, and the color developed with potassium thiocyanate. The curve (Fig. 2) was prepared by substituting standard solutions of pure ferric iron for the blood filtrate, and plotting the values.

Procedure. Introduce 1 c.c. of well mixed oxalated blood into a 100 c.c. volumetric flask, add 4 c.c. of concentrated H_2SO_4 (shaking well all the time), and allow to stand at least one to two minutes. Add 4 c.c. of a saturated solution of potassium persulfate ($K_2S_2O_8$)* and shake. Cool under the cold water

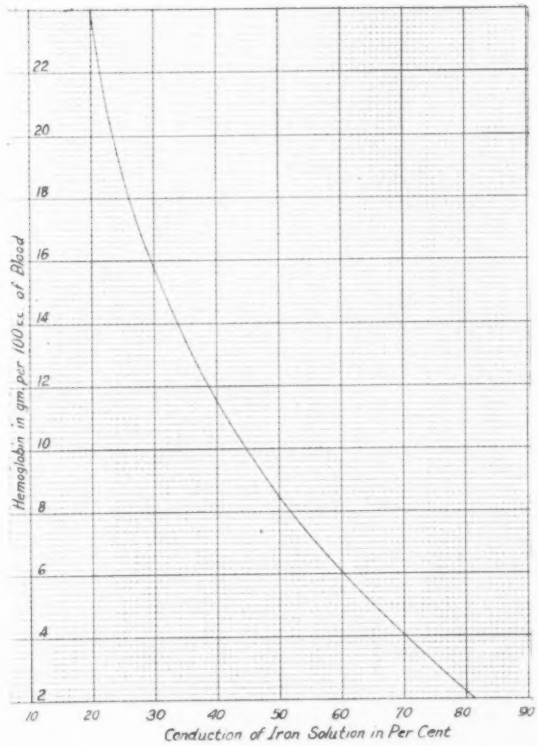

Fig. 2.—The light conduction of ferric thiocyanate solutions. The conduction of ferric thiocyanate solutions of varying concentrations has been plotted against the equivalent hemoglobin value (as calculated from the known iron content).

tap, and add (cautiously to prevent foaming) distilled water to within 5 to 10 c.c. of the mark. Then add 4 c.c. of 10 per cent NaOH, dilute to the mark, and mix well.

Filter through iron-free filter paper (Whatman No. 40 is satisfactory). Transfer 20 c.c. of this filtrate† to a 25 c.c. volumetric flask, add 1 c.c. of potassium persulfate, and dilute to the mark with approximately 3 N potassium thiocyanate.‡ Mix well.

*Prepared by adding about 4 gm. of pure potassium persulfate to 100 c.c. of warm distilled water, shaking well, and allowing the excess salt to settle out.

†If standard iron solutions are used, the procedure is started at this point.

‡Prepared by dissolving 291 gm. of pure KCNS in distilled water, and making the volume up to 1000 c.c. Filter if not entirely clear.

The colorimeter should have been turned on about fifteen to twenty minutes before starting the experiment to allow it to "heat up." Fill the sample cup with pure water, adjust the line voltage to some convenient figure (112) by means of a voltage regulator, and then set the current coming from the instrument at 0.40 milliamperes (the latter is imperative). Now insert a No. 47 Wratten filter (2 inches square), and set the instrument at 100. Balance the two cells, keeping the entering voltage at 112 volts.*

Now fill the cup (it is convenient to have two cups, one for the blank and one for the unknown sample) with the sample prepared above, and determine the percentage of conduction. By using the curve in Fig. 2, the hemoglobin is read off directly, and the blood iron is found by multiplying by 0.00335.

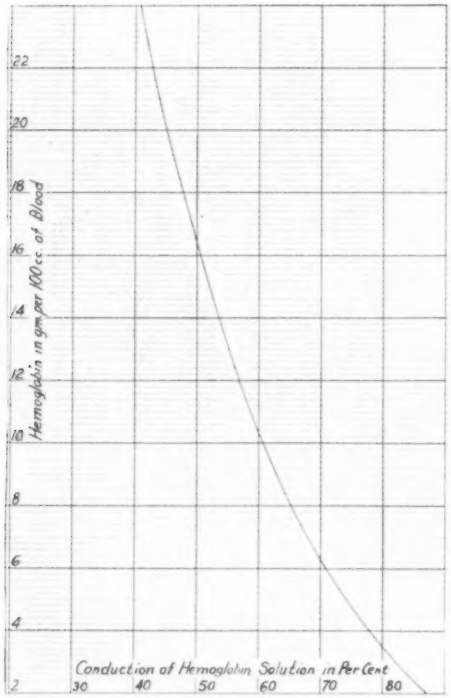

Fig. 3.

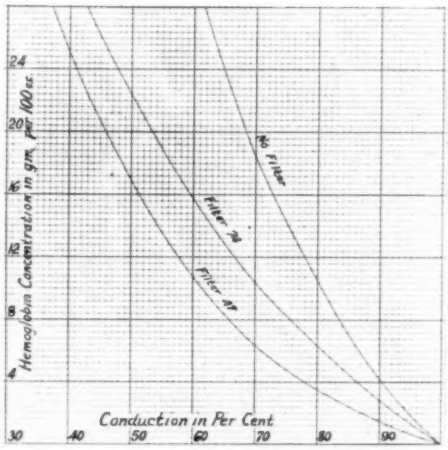

Fig. 4.

Fig. 3.—The light conduction of hemoglobin solutions. The conduction of whole blood diluted 1:400 with distilled water is plotted against the actual hemoglobin concentration.

Fig. 4.—Comparison of the conduction curves of different concentrations of hemoglobin without a filter and with Wratten filters 74 and 47.

Direct Method for Hemoglobin.—Procedure. Using a 0.05 c.c. pipette (graduated to contain),† transfer 0.05 c.c. of oxalated or finger-tip blood to exactly 20 c.c. of distilled water,‡ and wash the pipette with the solution

*When filters are not used, the current in the colorimeter can be kept at 0.40 milliampere by the rheostat on the machine. However, when a filter is employed, the current generated by the photoelectric cells is too small to accurately measure, and a constant cell output is best maintained by first adjusting the current from the electric cell to some value (0.40 milliamp.), noting the voltage, and keeping the entering voltage at this figure all through the determination.

†We obtained such pipettes from the Fisher Scientific Co. A larger pipette can be used just so the dilution is 1:400. Care must be taken not to draw the blood very far past the 0.05 c.c. mark, as the blood sticking to the side of the pipette introduces an appreciable error.

‡Sheard and Sanford[2, 4] diluted the blood 1:200 with 0.1 per cent Na_2Co_3 to prevent precipitation of proteins. We have found a dilution of 1:400 to give more consistent and reproducible results. At this dilution the slight haziness of any precipitated proteins is negligible, and a special solution for dilution seems to be unnecessary.

three or four times by sucking it up into the pipette. Do not blow through the solution. This makes a dilution of 1:400. Allow to stand one to two minutes (it may stand four hours), and read the conduction in the photoelectric colorimeter. The instrument is previously set at 100 with water in the cup, using a No. 47 filter as before. The current from the cells is kept at 0.40 milliamperes (without the filter) by adjusting the entering line voltage to the correct amount. From Fig. 3 the hemoglobin is obtained directly. The whole procedure takes about five minutes.

Types of Filters.—The Wratten filter recommended by Kennedy[13] for hemoglobin measurements (using a direct dilution of blood) is No. 74—a green filter. Experimentation, however, showed the blue No. 47 filter to be distinctly better. In Fig. 4 is shown a comparison between the transmission of plain diluted hemoglobin and that when Nos. 74 and 47 filters are used. It is obvious that a larger amount of the scale of absorption is used with the blue filter. Another point in favor of the 47 filter is that it conducts *much* more light, and is, therefore, more sensitive to changes in conduction, giving more accurate readings. In fact, we have found this filter to be best in nearly every determination where red or reddish colors are to be matched.

TABLE I

COMPARISON OF HEMOGLOBIN VALUES BY THE THREE METHODS

SAMPLE	HEMOGLOBIN BY BLOOD IRON METHOD IN GM. PER 100 C.C.	PHOTOELECTRIC METHOD FOR HEMOGLOBIN		SAHLI-HELLIG METHOD FOR HEMOGLOBIN	
		VALUE IN GM. %	ERROR IN GM. %	VALUE IN GM. %	ERROR IN GM. %
1	17.5	18.0	+0.5	15.8	−1.7
2	15.4	15.6	+0.2	16.3	+0.9
3	15.0	15.0	0.0	14.6	−0.4
4	15.4	15.0	−0.4	15.2	−0.2
5	13.4	13.2	−0.2	13.6	+0.2
6	17.0	17.0	0.0	17.0	0.0
7	15.8	16.2	+0.4	16.7	+0.9
8	17.5	18.0	+0.5	17.8	+0.3
9	16.4	16.3	−0.1	17.2	+0.8
10	17.3	17.5	+0.2	15.6	−1.7
11	15.3	15.4	+0.1	15.8	+0.5
12	14.7	14.2	−0.5	15.2	+0.5
13	16.6	16.3	−0.3	16.8	+0.2
14	16.6	16.2	−0.4	16.2	−0.4
15	15.2	14.8	−0.6	15.6	+0.4
16	15.5	15.4	−0.1	15.2	−0.3
17	5.3	5.2	−0.1	5.4	+0.1
18	12.3	12.0	−0.3	12.6	+0.3
19	13.4	13.3	−0.1	14.1	+0.7
20	13.5	13.6	+0.1	13.4	−0.1
21	15.4	15.1	−0.3	14.1	−1.3
22	15.9	16.6	−0.7	15.0	−0.9
23	14.8	14.4	−0.4	14.0	−0.8
24	14.4	14.0	−0.4	13.0	−1.4
25	11.1	11.3	+0.2	12.2	+1.1
26	14.2	15.0	+0.8	14.6	+0.4
27	13.9	14.0	+0.1	14.0	+0.1
28	6.4	6.6	+0.2	6.8	+0.4
Average	14.5	14.5	0.36*	14.45	0.81*

*These figures were arrived at by squaring each error and taking the square root of the average of the squared errors. We have referred to this value as the "average error."

Comparison of Hemoglobin Values.—In Table I is given for comparison the hemoglobin of 28 normal and pathologic subjects using (a) the blood iron

method (as standard of reference), (b) the direct photoelectric method just described, and (c) the Sahli-Hellig hemoglobinometer (prism type). The blood iron determinations were made in duplicate, and the Sahli determinations carried out by one very skilled in handling the instrument. The mean values for all, together with the "average error" are given at the end of the table.

DISCUSSION OF RESULTS

It can be seen by the table that the agreement of all three of the hemoglobin methods is quite close. Part of the reason for the Sahli-Hellig values following those for the blood iron so well was due to the determinations being made by one very skillful in handling the instrument. In the hands of the average individual (and in our own hands), such excellent agreement is not obtained. Notwithstanding the close correlation of the Sahli-Hellig and blood iron figures, the "average error"* (blood iron values taken as a standard of reference) is more than twice as large with the Sahli-Hellig as with the direct photoelectric method. In other words, the photoelectric method is considerably more accurate. The Newcomber disc method was tried in a few cases, but since the values did not check those for the blood iron as well as those from the Sahli, the values are not included.

The advantages of the direct photoelectric method are chiefly as follows: (1) It is very rapid. For a single determination it takes as much time as the Sahli type of instrument, but with a series of determinations it saves considerable time. (2) The readings need not be made for three to four hours after the dilution with water, and they can be made at once. The blood itself can stand at least two or three hours with no appreciable change in the hemoglobin values. (3) Errors arising from waiting too long or too short a time (and such are present in all rapid acid hematin methods) are entirely eliminated. (4) The values are reproducible—to the third significant figure with accurate measurement of the blood and handling of the instrument. Of course, it is obvious that such an expensive piece of apparatus could not be purchased for the determination of hemoglobin alone, but previous work (and the rest of the papers of this series) shows the advantages of using a photoelectric colorimeter in nearly all pathologic chemistry methods.

A point that immediately arises is whether or not the curve given for hemoglobin (Fig. 3) could be used by another operator on another instrument with another filter, or would each laboratory worker have to construct his own curve. It is true that the latter method would undoubtedly be safest and best, but since it occupies considerable time it is our belief that such effort may be unnecessary. While we have not had an opportunity to try this curve on another instrument, the fact that the null-point method of making the readings is employed makes it almost certain that there will be little difference between similar instruments.† Two different filters (same

*The "average error" was found by taking the square root of the average of the squares of the individual errors. This error (figure) actually means that in two-thirds of all determinations made, the actual error in grams will be less than this amount.

†Exton[9] states that the plotted curve can be used with different instruments (his own type), with an error of less than 5 per cent. We believe this will also hold true with the instrument that we used.

number) gave the same values; likewise different cups (these cups are guaranteed by the manufacturer to be within 0.005 cm. of 1.0 cm. in thickness, making the greatest possible error less than 0.5 per cent). Of course some practice is necessary to make the most accurate readings, but as soon as one becomes familiar with the instrument he is soon impressed with the ease of manipulation and high degree of accuracy in the duplication of conductivity readings. One thing is certain: a fairly accurate curve for hemoglobin can be constructed by each individual in a fairly short time, in the same manner that we made ours.*

In regard to the blood iron method, considerable experimental work showed us that the use of a curve (Fig. 2) gives much more accurate results than the use of simultaneous standards. In fact, much of our earlier work was discarded for the reason that the iron was determined by simultaneous comparison with standard solutions. It is certainly a much more rapid procedure, and always gives results that check within the limits of the accuracy of the measurements. We have found Wong's method quite satisfactory, but we believe that some of the newer methods (notably that of Coombs[14] or of Niederhäusern and Ferrarini[15]) may have additional advantages and even be more accurate.

SUMMARY

1. A rapid method for the determination of hemoglobin is described, whereby the light conduction of a watery solution of whole blood is determined with a photoelectric colorimeter, and the concentration of hemoglobin is read directly from a graph. The method seems to be more accurate than the usual acid hematin methods, and is much more rapid where a large number of samples are being analyzed.

2. The determination of blood iron by the use of the photoelectric colorimeter is also outlined, and a curve given to obviate the use of standard solutions and to increase the accuracy of the method.

3. A No. 47 Wratten filter was found to be most useful in both hemoglobin and blood iron determinations.

We wish to take this opportunity to thank Mr. Fabry L. Hawk for making the Sahli-Hellig hemoglobin determinations that are given in this work.

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*The construction of the curve in Fig. 3 is carried out in this manner. The blood iron is determined on several samples of blood. Then each sample is subjected to a number of simple watery dilutions, ranging from 1:300 to 1:2000, and the conduction of light through each dilution determined. The 1:400 dilution is taken to equal the concentration of hemoglobin shown by the blood iron measurement (for example, 16 gm.). Then the 1:800 dilution would be 8 gm.; the 1:600, 12 gm., etc. Plotting the dilutions of three or four bloods is sufficient to construct a satisfactory curve.

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THE DETECTION OF ACETONE AND ACETOACETIC ACID IN URINE*

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NUMEROUS qualitative tests (Lange, Le Noble, Rothera, Rantzman) have been proposed for the detection of acetone and acetoacetic acid in urine based on the reaction of these compounds with sodium nitroprusside in the presence of ammonia to form a deep permanganate-violet color. When performed directly on urine, a red color is given by creatinine, which disappears on the addition of acetic acid. Thus, three steps are involved in a direct qualitative determination of acetonuria: (a) addition of nitroprusside solution, (b) acidification (to avoid a false positive due to creatinine), and (c) alkalinization with ammonia.

In laboratories where determinations of acetone bodies are performed routinely on all urines, the use of strong aqua ammonia is extremely undesirable. Not only are its fumes highly noisome and noxious to the technician, but the danger of contamination of blood reagents and staining solutions by the volatile ammonia gas is ever present.

Our new method for the detection of acetone and acetoacetic acid is based on the same sensitive and specific color reaction with nitroprusside. However, instead of ammonia, a strongly alkaline organic amine, monoethanolamine, is used. The procedure is quite simple:

Place 10 c.c. of unfiltered urine in a test tube, add one drop (0.05 c.c.) of a saturated aqueous solution of sodium nitroprusside, 1 c.c. of a 15 per cent aqueous solution of monoethanolamine, and mix. In the presence of acetone or acetoacetic acid, the entire contents of the tube will turn to a deep permanganate-violet color within sixty seconds. With a little experience, the

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technician will soon be able to estimate the concentration of acetone bodies semi-quantitatively by the intensity of color (as, for instance: trace, one plus, two plus, etc.).

This procedure will detect 0.054 per cent of acetone in urine and 0.040 per cent of acetone in aqueous solution within one minute. The normal range of creatinine concentrations in urine (between 0.05 per cent and 0.25 per cent) does not give any color by this method, while a saturated solution of creatinine in urine gives a brownish-orange color which can hardly be mistaken for the deep permanganate-violet color given by acetone and aceto-acetic acid.

Acetone determinations were performed by this method on a series of 5,000 urines (among which were 521 positive acetones) and compared with the Le Noble reaction simultaneously performed. In every case absolute qualitative agreement between the two procedures was observed.

A COMPARISON OF METHODS FOR THE DETERMINATION OF PHENOL RED IN GASTRIC CONTENTS*

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IN THE usual methods of gastric analysis it is not possible to determine how much of the fluid of the test meal remains in the stomach to dilute the acid secreted by the stomach, nor is it possible to determine the acidity of the total secretions entering the stomach. Wilhelmj, O'Brien, and Hill[1-3] pointed out these and other deficiencies of the commonly used test meals, and introduced an improved test meal consisting of a specially prepared 2 per cent Liebig's meat extract solution containing 15 mg. of phenol red per liter. When the per cent of phenol red is determined in each gastric sample, it is possible to say just how much of the sample consists of secretions mixed with the meal and how much is fluid of the original test meal. It is also possible to determine the acidity of the total secretions entering the stomach.

In the method of Wilhelmj, O'Brien, and Hill, the per cent of phenol red in each gastric sample is determined colorimetrically by comparing the gastric samples with a sample of the original test meal similarly treated. In order to remove organic material which would interfere with the colorimetric determination, equal volumes of the original test meal and of each of the gastric samples are treated with 20 per cent sodium tungstate and 1.33 normal sulfuric acid, centrifuged and alkalinized.

When the fractional method of gastric analysis is used, it is usually found that the final samples are so dilute that it is impossible to make an accurate colorimetric determination of the per cent of phenol red present. In order to correct for the excessive dilution of the final samples, an aliquot fraction of the

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sample is accurately mixed with an equal volume of the original test meal, and the per cent of phenol red determined on the mixed sample; by a simple correction the per cent present in the original gastric sample can then be calculated.[1] When this is done, the per cent of phenol red in the mixed sample can never be below 50 per cent. This method was carefully checked and found to be very satisfactory.

It was also found that when the gastric samples contained large amounts of bile, it was not always completely removed by the treatment with sodium tungstate and sulfuric acid, so that after alkalinization the gastric samples would have a definite yellow tinge when compared in a colorimeter with a sample of the original test meal. In order to correct for this, a few small crystals of picric acid are added to about 2 c.c. of the test meal standard, and a few drops of the pierated standard mixed in the colorimeter cup with the untreated standard, until the yellow tinge of the standard matches that of the gastric sample. A series of carefully controlled experiments showed that this procedure is entirely satisfactory.

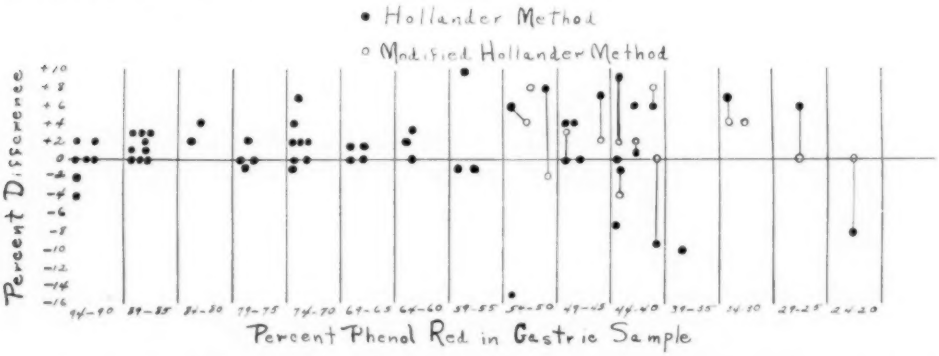

Fig. 1.—The gastric samples were grouped according to decreasing percentages of phenol red present. The line represents perfect agreement between the two methods. The solid dots show the percentage difference between the two methods when the original Hollander method was used, and the circles the percentage difference when the Hollander method was modified by correcting for excessive dilution of the gastric samples.

Recently Hollander, Penner, and Saltzman[4] have introduced another method for determining the per cent of phenol red in gastric samples. Organic material is precipitated by zinc hydroxide and the samples alkalinized, and the excess zinc hydroxide removed with sodium phosphate. In their method no correction is made for excessive dilution of the samples. They state that all bile is removed, so that no correction is necessary for a yellow tinge of the gastric samples.

In order to compare the two methods, we have performed a series of twenty fractional gastric analyses comprising 65 half-hour gastric samples in which the per cent of phenol red was determined in each gastric sample by both methods. The 2 per cent Liebig's extract test meal[1] was used in all. Thirteen experiments were performed on normal dogs, three on dogs in which we had produced an experimental hypersecretion of acid, and four were on a dog having a gastroduodenostomy, so that large amounts of bile-stained duodenal secretions entered the stomach.

The results are shown in Fig. 1. It is seen that down to approximately 50 per cent of phenol red in the gastric samples, the agreement between these two methods is usually quite satisfactory, especially when it is remembered that a variation of ±2 per cent is the maximum degree of accuracy possible (in our hands) in matching the phenol red color. Below approximately 50 per cent, the agreement between the two methods is not satisfactory. This is due primarily to the fact that accurate colorimetric determinations cannot be made when the standard solution is two times or more as strong as the unknown solution. Theoretically, according to Beer's law, there should be a direct proportionality between the per cent of phenol red in the sample and the colorimeter reading through all ranges of dilution; practically, however, this is seldom the case. Folin[5] emphasized that accurate colorimetric readings cannot be made when one solution is more than 1.5 times as strong as the other, a fact which is generally accepted by most investigators.[6-8] Some of the samples prepared according to the Hollander method were so excessively dilute that it was impossible to make a comparison with the standard. Dilution of the standard with distilled water, so that it closely approximates the unknown, is unsatisfactory, because the color of both is then too faint for accurate matching.

When the Hollander method was modified by mixing the very dilute gastric samples with an equal volume of the original test meal, the agreement between the two methods was improved in 8 out of 12 samples. The importance of correcting for excessive dilution is clearly shown by the fact that when no correction was made in the Hollander method, 76 per cent of the samples agreed within ±4 per cent by the two methods; but when the correction was made in the Hollander method, 88 per cent of the samples agreed within ±4 per cent.

When the dog with a gastroduodenostomy was studied, it was found that the Hollander method often failed to completely remove the bile so that the alkalinized gastric samples had a definite yellow tinge when compared with the standard test meal sample. In order to have made a satisfactory colorimetric reading, it would have been necessary to apply the picric acid correction to the standard solution. During the course of these studies it was again demonstrated that the picric acid correction gives highly satisfactory results.

SUMMARY

1. A comparison of the Hollander and Wilhelmj methods for determining the per cent of phenol red in gastric contents shows that down to approximately 50 per cent of phenol red in the gastric samples, the agreement between the two methods is usually satisfactory. Below approximately 50 per cent discrepancies are frequent and marked. When the Hollander method is modified by correcting for excessive dilution of the gastric samples, the agreement is greatly improved.

2. Both methods may at times fail to entirely remove the bile from gastric samples containing excessive amounts, and it may be necessary to apply the picric acid correction.

3. When the Hollander method is modified to correct for excessive dilution of the samples, the two methods are about equally reliable.

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A NOTE ON SOME PRESUMPTIVE TESTS FOR BENCE-JONES PROTEIN*

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THERE is need for a procedure that will detect Bence-Jones protein during the routine examination of urine, or lacking this, one which will serve as a presumptive test for this substance and indicate further confirmatory tests. Bence-Jones proteinuria is rare, and routine detailed examination for it would be too time-consuming, but when it does occur, it is highly desirable to detect its presence. For it to manifest its characteristic precipitation at about 60° C., re-solution at boiling and reappearance when cool, the pH and salt concentration must have been suitably adjusted.[1] The inadequacy of the usual tests for proteinuria for demonstration of Bence-Jones protein at all consistently has been admirably proved by Osgood and Haskins.[2] These authors recommend two procedures: (a) To 5 c.c. urine, add 1 c.c. 50 per cent acetic acid and 3 c.c. 30 per cent (saturated) sodium chloride. They believe a precipitation appearing at room temperature "strongly suggests the presence of Bence-Jones protein," though a precipitate may occur when the urine contains 38 mg. per cent globulin. Their impression is that such urines are uncommon. (b) To 1 c.c. urine, add 0.1 c.c. 20 per cent sulphosalicylic acid solution. All proteins precipitate, but Bence-Jones protein redissolves at the boiling point.

The first presumptive test has found a place in several tests.[3-5] When we employed it in several instances of unexplained proteinuria, we were forced to the conclusion that it would give a positive result with nearly any urine in which protein could be found. In an effort to substantiate this impression quantitatively, we have analyzed urines from a representative group of renal diseases.

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The total protein was precipitated by 10 per cent trichloracetic acid; globulin by half saturation with ammonium sulfate, the supernatant fluid serving for albumin estimation. The amount precipitated by salt and acetic acid in the above concentrations was also determined. All precipitates were well washed, the second washing uniformly showing no color with Folin's phenol reagent, and the various protein fractions were determined colorimetrically by the method of Andersch and Gibson.[6]

TABLE I

NO.	CLINICAL DIAGNOSIS	TOTAL PROTEIN MG. %	ALBUMIN MG. %	GLOBULIN MG. %	PPT. BY NaCl AND ACETIC ACID	DILUTION STILL PPT.	GLOBULIN IN FINAL DILUTION MG. %
1	Acute nephritis	102	37	65	101	1:20	3.2
2	Amyloidosis	371	260	111	306	1:50	2.2
3	Pre-eclamptic toxemia	348	320	28	274	1:30	0.93
4	Febrile albuminuria	118	86	32	86	1:15	2.1
5	Congestive failure	202	180	22	175	1:15	1.5
6	Hypertension	179	169	10	174	1:10	1.0
7	Congestive failure	121	115	6	112	1:10	0.6
8	Congestive failure	210	190	20	191	1:20	1.0
9	Pre-eclamptic toxemia	795	708	87	780	1:70	1.2
10	Chronic nephritis	150	120	30	122	1:32	0.94
11	Subacute nephritis	695	545	150	675	1:80	1.9

Our results are tabulated. Each urine was diluted serially, and the highest dilution which gave a precipitate with salt and acetic acid was recorded. We calculated the amount of globulin which was present in this dilution; dilutions containing from 1 to 3 mg. per cent still reacted positively. As may be seen from the table, urine containing over 38 mg. per cent globulin is by no means uncommon. The bulk of the precipitate in each instance was albumin.

Our experience with the second procedure was more satisfactory, and we believe it conforms more nearly to the requirements of a presumptive test for Bence-Jones protein. The salt and acetic acid method is still valuable as a means of precipitating the protein for removal of other urinary constituents;[4] precipitation with it, however, is not suggestive evidence for the presence of Bence-Jones protein.

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COMPARATIVE STUDY OF THE LAUGHLEN TEST FOR SYPHILIS*

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SINCE Wassermann[1] first applied the principles of complement fixation to the diagnosis of syphilis, attempts have been made to simplify the test without sacrificing sensitivity and accuracy. The tests suggested have for the most part been only partially satisfactory, and the search continues for a test which is simple and inexpensive and yet of sufficient accuracy to warrant its use routinely on all patients. The need for such a test becomes more apparent when it is realized that the ravages of syphilis seem to be increasing in spite of our increased knowledge of the disease. This situation can only be rectified by a more careful examination of all individuals, and the popularization of blood tests for the detection of the disease. Increased blood testing, on the other hand, depends largely upon the facilities available and the price of the test to the individual. All new tests for syphilis must, therefore, present the following features: (a) it must be easily done; (b) it must be accurate, and (c) it must be economical. Any test which does not satisfy these criteria need not be considered in our search for more formidable diagnostic weapons with which to fight the disease.

Recently, two new methods for the detection of syphilis have been offered—one is the Laughlen[2] test and the other is the Ide[3] test. Both of these are more easily performed than most approved serologic tests for syphilis, and both of them are more economical than other serologic tests. It becomes necessary then to determine the accuracy and sensitivity of these tests, and it is with this problem that the present communication deals. Only the Laughlen tests will be discussed at this time. The result of the Ide tests will be published later.

It is not a simple matter to treat these various methods in an exactly comparable manner. The tests with which the staff is familiar will always appear in a slightly better light than a newly acquired test. This is further evidenced by the fact that the originator of a method usually gets a higher percentage of positive results than other laboratories using the same method.

The method of obtaining patients also plays a role in the end results. Patients should not be selected for such a study as this, and the number tested should be large.

PROCEDURE

It was decided to do a thousand cases by Kahn[4] and Kline[5] tests, these being the serologic tests done in the laboratory routinely. On the same serum, the Laughlen test was to be performed by an individual not of the regular personnel of the serology laboratory. As far as possible, all the tests were done by

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one of us (J. E. G.). Two hundred tests were done as a preliminary chiefly to become accustomed to the test and to work out a routine technique which would give the necessary speed to allow the completion of 40 to 60 tests in a reasonable time. When these were finished, the thousand routine blood tests were run; that is, every blood which came to the serology laboratory for a Kahn and a Kline was also subjected to the Laughlen test. When the thousand tests were completed, they were checked against the Kahn and Kline results.

TECHNIQUE OF THE LAUGHLEN TEST

The test is extremely simple, but requires careful attention to details. The test may be done on ordinary microscope slides, two tests to the slide. The ordinary medicine droppers have been found very satisfactory for the measuring of both serum and antigen. The serum may be inactivated or not. In fact, the test may be done on whole blood, but interpretation is much more difficult if whole blood is used. The actual test is done by mixing one drop of serum and one drop of antigen on a clean slide and mixing the two by gentle rotation or jarring. The specimen should be examined every few minutes by indirect light for the presence of clumping which appears as tiny but definite red specks in the cloudy mixture. If clumping occurs in the first two minutes, the serum is strongly positive; the less strongly positive sera take longer to produce the characteristic reaction. Any clumping which occurs after ten minutes or as the specimen begins to dry must be ignored. A known positive serum and negative serum must always be run as a control. The specimen may be examined under the microscope, although after a little practice this is not necessary.

PREPARATION OF THE ANTIGEN

The details of this procedure have been given by Laughlen in the *Canadian Medical Association Journal*, and will only be briefly summarized here. The antigen used by us was supplied through the courtesy of the Lederle Laboratories.

The preparation of the antigen may be divided into four steps: (1) preparation, (2) modification, (3) dilution of the modified antigen, and (4) standardization of the antigen to a certain sensitivity.

The antigen is prepared as is the Kahn antigen and is cholesterolized by adding 6 mg. cholesterol to each cubic centimeter of antigen. It is then modified by the addition of scharlach R to saturation and the further addition of tincture of benzoin compound. The modified antigen is then diluted by the addition of 5 c.c. 1.5 per cent saline (at 50° C.) to each 0.5 c.c. of antigen. If the antigen is satisfactory at this stage, it will be opaque and the particles will not settle out in twenty-four hours. This, then, is the stable inactive antigen and, before it can be used, it must be activated by the addition of an electrolyte. The amount to be added must be determined by the use of positive serum, a sufficient amount of antigen being added to produce clumping in from one to two minutes, with a known strongly positive serum. When this is determined, a comparable amount of electrolyte (10 per cent solution sodium chloride) may be added to the stable antigen as needed.

The sensitized antigen will only be found reliable for about seven days, and the quantity prepared should not exceed that which will be used in this period of time.

RESULTS

It is rather difficult to interpret the results of various tests for syphilis, since no test is infallible. Every test so far devised is capable of giving both false positives and false negatives. The fact that, not infrequently, an individual may be a syphilitic and present no physical findings further complicates the matter.

TABLE I

	LAUGHLEN	KAHN	KLINE
Positive	138	142	133
Negative	867	858	867

TABLE II

	LAUGHLEN	KAHN	KLINE
False negative	18	2	8
False positive	24	1	2

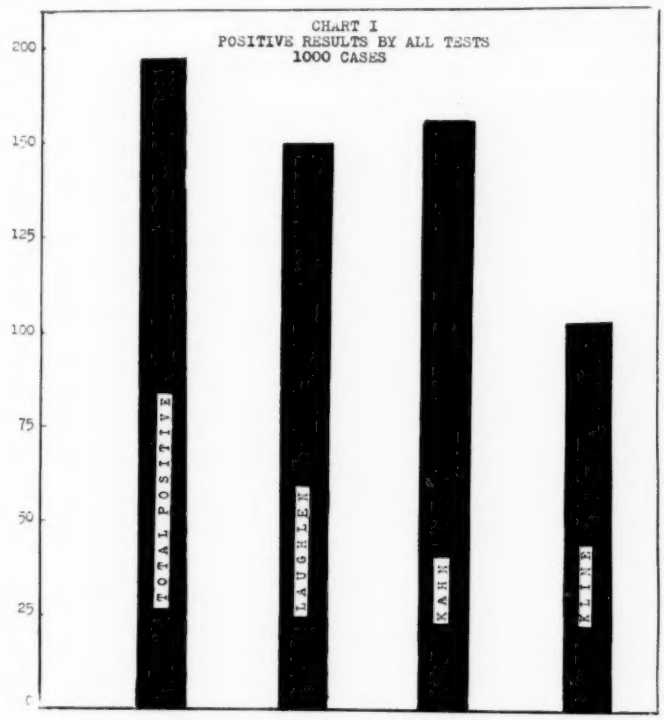
The Kahn test was the most accurate test in our hands, giving only one false positive reaction and two false negative reactions in 1,000 cases. The term, false positive, is here used to denote a positive serology by only one test in an individual who gives neither history nor physical findings consistent with diagnosis of syphilis. A false negative is, of course, the absence of positiveness by one test when other tests are positive and the patient is known to be a syphilitic. Chart I shows graphically the positive results. Table I gives the total number of

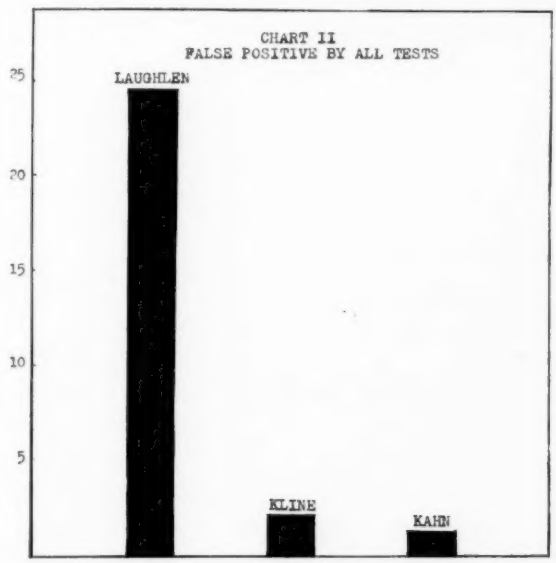
TABLE III

ANALYSIS OF FALSE POSITIVES BY THE LAUGHLEN TEST

NUMBER	CLINICAL FINDINGS
12988	Gonorrhea, positive Neisserian fixation test; no findings suggestive of syphilis
12451	Chronic cervicitis (gonorrheal?); no findings suggestive of syphilis
12279	Gonorrhea; severe secondary anemia; positive Neisserian fixation test
5631	Pregnancy; pyelitis
12524	Cardiac; negative for syphilis
12918	First trimester of pregnancy
7222	Chronic cervicitis; negative for syphilis
13062	Cervicitis, cystocele, and rectocele; negative for syphilis
12229	Far-advanced tuberculosis
12743	Miliary tuberculosis
12423	Hyperthyroidism
12571	Fractured skull; no evidence of syphilis
12511	Appendicitis and cervicitis
12742	Sinusitis
12945	Parkinson's syndrome; no evidence of syphilis
12853	Arthritis; no evidence of syphilis
12936	First trimester of pregnancy; no evidence of syphilis
13025	Far-advanced tuberculosis
11156	Peptic ulcer
12715	Postoperative parotitis
12034	Gonorrheal salpingitis; no evidence of syphilis
H. H.	Blood donor; no evidence of syphilis
P. L.	Blood donor; no evidence of syphilis
M. W.	Blood donor; no evidence of syphilis

positive tests obtained by the various methods used and the total number of positives by each test. Table II gives the number of false positive and negative tests by the various methods. The Laughlen test was less efficient than either

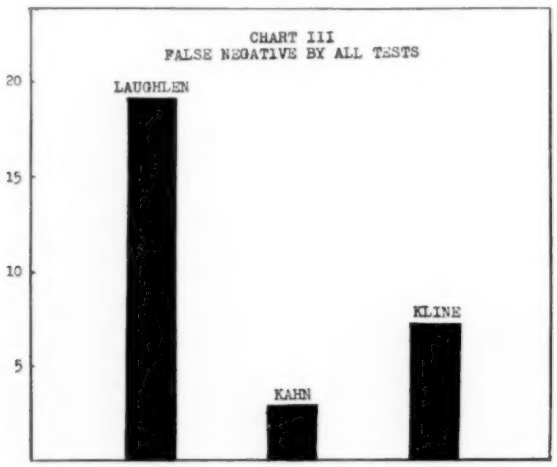




the Kahn or Kline. Table III gives an analysis of the false positive by the Laughlen technique, and Chart II gives the graphic representation of these. It will be noted that many of the false positives were obtained from individuals

with pelvic disease or pregnancy, while in several instances, the false positives were found in individuals suffering from advanced tuberculosis. Each patient who gave a false positive by the Laughlen test was re-examined clinically to rule out or discover a syphilitic history or lesion. In no instance did we succeed in doing this.

Table IV and Chart III present an analysis of the false negative obtained by the Laughlen technique. In two patients, the Kahn was the only test positive; but it will be noted that both of these patients had had a great deal of anti-syphilitic therapy, and when their histories were reviewed, it was found that



they had had positive Wassermann and Kline tests at the onset of their therapy. All the other cases, in which the Laughlen was falsely negative, had both Kahn and Kline tests positive. The Laughlen test was negative, when other tests were positive, in only one untreated case. We are, therefore, of the opinion that the Laughlen test is more easily reversed as a result of therapy than are the other

TABLE IV

FALSE NEGATIVES BY THE LAUGHLEN TEST

NUMBER	KAHN	KLINE	REMARKS
12624	Positive	Positive	Chancre forty years ago; treatment at intervals
340	Positive	Negative	Under continuous treatment for two years
7588	Positive	Positive	Treatment for three years
327	Positive	Positive	Treatment for two years
785	Negative	Positive	Treatment for two years
12648	Positive	Negative	Treatment for one month
10690	Positive	Positive	History of syphilis for forty years, sporadic treatment
12266	Positive	Positive	Treatment for four years; history of chancre
266	Positive	Positive	Treatment for four years, serology variable from negative to positive
12425	Positive	Positive	Treatment for four years; had gumma
1950	Positive	Positive	Treatment for two years
196	Positive	Positive	Treated for two years; chancre six years before treatment
12139	Positive	Positive	Treated for two years; Neisserian fixation also positive
12233	Positive	Positive	Congenital, treated for four years
1553	Positive	Positive	Variable serology; has been under treatment for four years
12602	Positive	Positive	Under treatment for a year
12462	Positive	Positive	No history of chancre; no therapy
P. V. G	Positive	Positive	Under treatment two years; tertiary syphilis

tests. This question is now being studied more carefully in a series of patients under treatment at the present time, and our conclusions will be presented when this work is finished.

DISCUSSION

It should be pointed out that as our experience increased the accuracy of the test increased. If we break up our series of a thousand cases into two groups, we find that 19, or about 80 per cent, of the false positive tests fall in the first 500 cases tested, while 8, or about 55.5 per cent, of false negatives occurred in the first 500 cases. These analyses of the cases, as two groups, strengthen our belief that the Laughlen test is quite accurate in untreated cases, but is more readily reversed by therapy than are our other tests.

The Laughlen test is a clean, quick, and economical test, with a fairly definite end point. The ability to determine the strength of the reaction on the basis of a time factor rather than on a quantitative basis is a distinct advantage, as there seems to be little reason to report any serologic test for syphilis by a series of plus signs. The test for syphilis is far better reported as negative, positive, or doubtful.

The Laughlen test, just as any other serologic test, requires a certain skill and training, and does not lend itself to use by everyone. The large number of tests, which is necessary to produce efficiency in even a trained worker, seems to be sufficient reason for not recommending it as a test to be casually used in general practice. It might well be used, we think, by the large laboratories because of its speed and economy when suitable controls can and will be done frequently. The Laughlen test seems to be slightly less accurate than the Kahn test and compares fairly well with the Kline test.

CONCLUSIONS

1. The Laughlen test is a simple, inexpensive, serologic test for the diagnosis of syphilis, with a sharp end point.

2. The Laughlen test is slightly less accurate than the Kahn test and compares fairly well with the Kline test.

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A PRACTICAL WORK KIT FOR PNEUMOCOCCUS TYPING: NEUFELD TEST*

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FOR the past two years we have endeavored at the City Hospital to identify all 32 types of pneumococcus which might appear in the course of routine diagnosis. Two different lots of sera have been employed, one from the Lederle Laboratories, the other from the Department of Health of New York City. The methods in use by the Health Service of the Commonwealth of Massachusetts have been followed with very slight modification.

Fig. 1.—Work kit with Lederle and Department of Health pools.†

Six initial tests are performed with Lederle sera in the following manner, using 6 clean, dry cover slips and 2 glass slides. The glass slides are marked off into 3 square areas each and labeled according to the diagram below:

1	2	3

Specimen Numbers

5, 7, 8	14, 18	1-32

Specimen Numbers

The first slide holds the tests with individual sera of types 1, 2, and 3, the second with pooled sera, the pools consisting of (a) 5, 7, and 8; (b) 14 and 18; and (c) 1 to 32. On a cover slip is placed a loopful of sputum or culture, then one or two loopfuls of type 1 serum, and a drop of blue dye solution. This is mixed well, using a little saline if necessary to keep it moist. The edge is then rimmed with a thin oil and the cover slip inverted upon the corresponding square. The same procedure is used, employing types 2, 3, and the pools. The preparations are examined under the microscope for swelling of the capsule, which indicates a positive reaction. If a test is positive in pooled sera, repeated tests are then made using individual sera corresponding to the pool. Strong

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agglutination sometimes occurs with or without "quellung" in pooled sera, and indicates probable presence of at least two individual type reactions. We have been unable to obtain type sera above 32 for diagnostic procedure.

Sera of the Board of Health are used as controls. Five pools, however, are used, since our experience has shown that the complete pool of 1 to 32 with these sera is not as efficient as the smaller pools. The pools are made up as follows: (a) 5, 7, 8, 14, 18; (b) 10, 11, 12, 13, 15, 17; (c) 4, 6, 9, 16, 19; (d) 20, 21, 22, 23, 24, 25; and (e) 27, 28, 29, 31, 32. The Lederle pool of 1 to 32 was used to cross check the smaller pools and to facilitate finding the less frequent types.

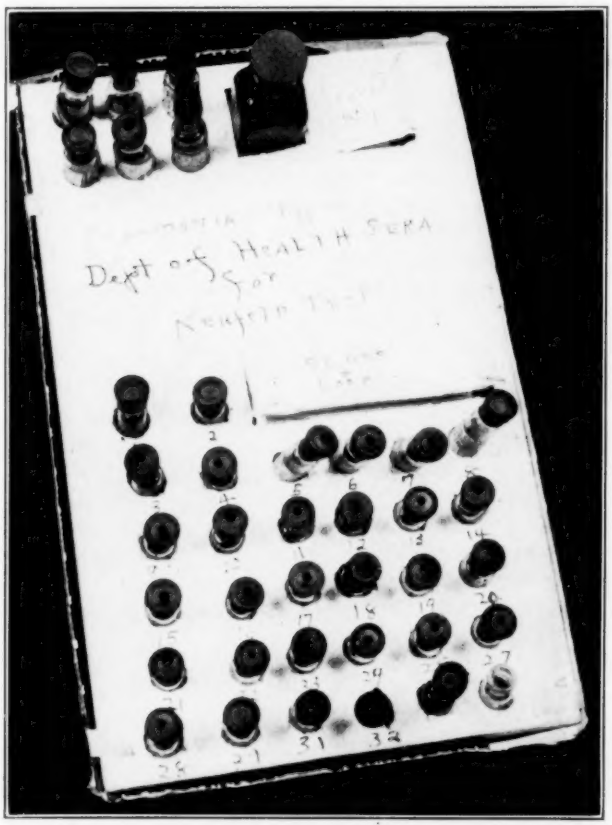

Fig. 2.—Work kit containing Department of Health sera controls. *A*, Pools, thin oil, pocket for cover slips; *B*, pocket for slides and loop; *C*, 32 individual sera.

Since therapeutic sera prepared by the Board of Health and by Lederle are both used in the hospital, it was thought pertinent to scientific accuracy that both diagnostic sets be employed. We have kept two separate boxes of the diagnostic sera, slightly different in dimensions and size of bottle and carefully labeled, so that no confusion can result.

If any question of identification arises, cultures and specimens are referred to the Research Laboratory, Pneumonia Division, Board of Health. During the past two years only one serum, type 5, lost its potency, and only two strains of pneumococcus were found which did not correspond to any of the recognized 32 strains. Statistical findings will be reported in a separate paper.

DEPARTMENT OF REVIEWS AND ABSTRACTS

ROBERT A. KILDUFFE, M.D., ABSTRACT EDITOR

MENINGITIS, Gonococcic, Branham, S. E., Mitchell, R. H., and Brainin, W. J. A. M. A. 110: 1804, 1938.

It seems likely that gonococcic meningitis is much more common than it is usually supposed to be. Hospital laboratories are usually much too busy to carry the identification of the causative microorganism to completion, and a gram-negative coccus of typical morphology which occurs intracellularly and extracellularly in the spinal fluid, and which is agglutinated by antimeningococcus serum, is usually assumed to be a meningococcus. Even when the study is carried further, definite criteria for the identification of the gonococcus are not well established and its identification is not easy. An absolute criterion is lacking; nevertheless, a careful study of cultures with consideration of all of their characteristics—type of growth, colony form, fermentation reactions, alkali solubility, and serologic reactions—will make an experienced worker feel quite sure of the identity of the strain in question.

Although gonococcic meningitis is usually secondary to gonococcic infection elsewhere, the case reported in this paper suggests that the gonococcus must be considered a potential cause of a primary meningitis without other gonococcic involvement.

PORPHYRIA, Studies in: Acute Idiopathic Porphyria, Turner, W. J. Arch. Int. Med. 61: 762, 1938.

Acute idiopathic porphyria may be associated with excretion of uroporphyrin I in excess of uroporphyrin III.

Excretion of metal-porphyrin complexes appears to be characteristic of acute idiopathic porphyria.

Most of the color of the urine in acute idiopathic porphyria is usually due to the presence of pigments which have been called urofuscin.

Acute idiopathic porphyria is a familial disease, probably inherited as a dominant Mendelian characteristic.

The acute manifestations are alleviated by intravenous calcium therapy.

MARROW, Culture of Human, in Pneumococcic Infections, Osgood, E. E. Arch. Int. Med. 62: 182, 1938.

Culture of human marrow makes possible a type of control which is attainable in animal experimentation or in clinical investigation. In human marrow cultures, sulfanilamide exhibits a slight bacteriostatic action on pneumococcic infections which is increased by an increase in concentration. Even 0.3 unit per c.c. of specific antipneumococcus serum is more effective against the type I pneumococcus than is sulfanilamide alone. Sulfanilamide plus any given dose of antiserum, less than the amount which will by itself reduce colony counts to nearly zero, is more effective than corresponding doses of antiserum alone. These effects do not depend chiefly on phagocytosis. The results support the view that sulfanilamide renders the organism more vulnerable to bactericidal substances present in the serum. If the results of these in vitro experiments on the interaction of therapeutic and noxious agents in the presence of living human cells are applicable to infections in human beings, sulfanilamide therapy should be of value in pneumococcic pneumonia and might delay death in pneumococcic meningitis, but it will not prove as effective as even small amounts of type-specific antiserum. If used in conjunction with antiserum, it should further lower the mortality with the present doses of antiserum, or should give an equally low mortality with smaller doses of antiserum.

The use of both sulfanilamide and therapy designed to introduce or to develop specific bactericidins should be investigated further as a possible effective treatment for infections which are relatively resistant to the action of sulfanilamide alone.

UROBILINOGEN: Excretion of Bile Pigment and Hepatic Function in Diseases of the Blood, Barker, W. H. Arch. Path. 62: 222, 1938.

The excretion of urobilinogen has been studied in 74 cases of blood disease. The average daily output of urobilinogen in the feces is regarded as an index of the rate at which erythrocytes are being destroyed in the body.

Abnormally high values for urobilinogen in the feces were obtained in the cases of hemolytic anemia and in most of the cases of pernicious anemia in relapse; these values returned to normal after splenectomy in the cases of congenital hemolytic jaundice and after adequate liver therapy in the cases of pernicious anemia.

Based on these observations, the conclusion is reached that destruction of blood is increased in pernicious anemia, even though the disease may not be primarily a hemolytic anemia.

The anemia occurring in association with leucemia is occasionally hemolytic. In rare cases of aplastic anemia, a hemolytic factor is apparently active; splenectomy in 2 such cases was followed by temporary improvement.

Transfusion of well-matched blood may at times be followed by a striking increase in the excretion of bile pigment, suggesting that the better part of the transfused blood is rapidly hemolyzed in such instances.

In 63 of the 74 cases of blood disease, the hepatic function was studied by one or more of three tests; the test for urobilinogen in the urine, the bilirubin excretion test, and the hippuric acid synthesis test. Evidence of hepatic dysfunction was obtained in over 50 per cent of the cases, suggesting that a disturbance in the function of the liver may play a significant role in the pathogenesis of many of the blood dyscrasias.

MENINGITIS, Tuberculous, Levinson Test in, Gleich, M. Am. Rev. Tuberc. 38: 239, 1938.

Of 30 cases of tuberculous meningitis all gave a positive Levinson test. In 76.6 per cent of these patients tubercle bacilli were found in the spinal fluid. Twenty per cent of the cases showed tuberculosis at autopsy. In one case tuberculosis was noted at autopsy and tubercle bacilli were demonstrated in the spinal fluid.

None of 13 cases of nontuberculous meningitis had a positive Levinson test. One will occasionally find cases of nontuberculous meningitis or patients with other brain lesions who give a positive Levinson test. If it is remembered to refrain from performing a Levinson test on the spinal fluid which is contaminated by blood, or where serum has been previously given intrathecally, such pseudopositive Levinson tests will be avoided.

GLUCOSE-TOLERANCE TEST, the One-Hour, Two-Dose, Cooperstock, M., and Galloway, J. M. Am. J. M. Sc. 55: 1221, 1938.

The one-hour, two-dose dextrose tolerance test, previously found to be a specific, time-saving, and convenient procedure for adults, was applied to infants and children.

The technique of the original test was modified to suit this age period. The micro-method for determination of the blood sugar was found to be a reliable additional simplification of the procedure.

One hundred and two tests were performed on 82 subjects, consisting of 30 controls, 16 patients with diabetes mellitus of varying degree and duration, and 36 patients with non-diabetic conditions.

The characteristics of the normal response to the one-hour, two-dose dextrose tolerance in a control group of subjects were found to be as follows: (1) a normal blood sugar level during fasting (average, 91 mg.); (2) a more or less steep rise at thirty minutes (10 to 63 mg.), and (3) a fall below the thirty-minute blood sugar level at the sixty-minute determination in 76.5 per cent of the tests, and an elevation above the thirty-minute level at sixty minutes in 23.5 per cent, the total rise in the blood sugar level in no instance exceeding the normal level during fasting by more than 80 mg.

Application of these diagnostic criteria to the group of patients with nondiabetic conditions did not yield any unaccountable deviations.

The one-hour, two-dose dextrose tolerance test performed for the group of diabetic patients revealed the following features: (1) a normal blood sugar level during fasting or one above the normal limits (120 mg.); (2) a more or less steep rise at thirty minutes; (3) a hyperglycemic level at sixty minutes, the total rise exceeding 80 mg., with an occasional exception in cases of early diabetes mellitus of the low threshold type, and (4) glycosuria in at least the sixty-minute specimen.

Several abnormal curves were demonstrated for patients with nondiabetic conditions associated with deviations of normal carbohydrate metabolism.

The employment of the one-hour, two-dose dextrose tolerance test for infants and children demonstrated the important features of specificity, timesaving, and convenience. All these considered, the test offers an improved method for estimating dextrose tolerance in subjects of this age period.

ANEMIA, Hyperchromic, in Infants, Stephenson, R. Am. J. Dis. Child. 55: 1141, 1938.

A study was made of 64 normal white American-born boys and girls, ranging from 3 months to 2 years in age, and living under the same conditions in an institution having good pediatric supervision.

There being no other known variable, half of the infants were each given 30 gr. of iron and ammonium citrates (about 340 mg. of metallic iron) daily; the control infants were each given 6 gr. of ferrous sulfate (about 78 mg. of metallic iron) daily. Therapy was continued for three months, the effects being checked by estimations of red cell count, hemoglobin, and red cell volume.

As indicated by increases in hemoglobin, the ferrous iron proved to be as effective as the ferric. Apparently 10 gm. of hemoglobin per 100 c.c. of blood is the optimal level to be expected from administration of these forms and doses of iron, continued for three months, for infants of this age period.

The two groups having reached much the same average hemoglobin level at the end of three months, one group was then withdrawn from iron therapy while the other group continued to receive supplemental iron for an additional three months. The trend of the hemoglobin level was downward in those infants whose medication was discontinued, whereas it was upward in those for whom medication was maintained.

It seems probable that the increases in hemoglobin were the result of generous supplements of iron alone rather than of the traces of copper which contaminated both iron compounds.

This study confirms the claim that seemingly normal babies, between the ages of 3 months and 2 years, have a borderline anemia, and demonstrates that their hemoglobin levels can be raised to about 14 gm. per 100 c.c. by treatment with adequate iron without added copper. Increases in the number and volume of red cells also result, though they are less marked. Improvements in the infant's color, activity, and resistance to infection are believed to result, though they are difficult to measure. In this investigation ferrous iron produced a maximum effect with much smaller doses than ferric, and it caused no gastrointestinal disturbance. Some supplemental iron appears to be needed, up to the age of 2 years at least, to maintain the hemoglobin at this optimal level; it is possible that less than 6 gr. of ferrous sulfate would be an adequate maintenance dose.

VITAMIN C., Urinary Output of Normal and of Sick Children, Bumbalo, T. S. Am. J. Dis. Child. 55: 1212, 1938.

A macrochemical modification, both easy and accurate, of the Harris and Ray method for the determination of cevitamic acid is presented.

The urinary excretion of cevitamic acid of a normal child on an average diet is found to range from 11.2 to 71.2 mg. per twenty-four hours, directly dependent on the vitamin C intake.

The results of the study suggest that there is some vitamin C deficiency in all forms of tuberculosis in children. It is suggested also that there is some vitamin C deficiency in all febrile conditions in children.

The method of determination used follows:

(Tillman's reagent: 0.05 per cent solution of 2.6-dichlorophenolindo phenol made acid by the addition of 2 drops of glacial acetic acid; 0.05 c.c. equivalent to roughly the amount of 0.025 mg. of cevitamic acid.)

From 2 to 15 c.c. of urine is brought to a final volume of 15 c.c. and then brought to a pH of 4.7 by the addition of 0.02 c.c. of glacial acetic acid, Congo red being used as an indicator. It is then titrated with Tillman's reagent to an end point at which a lilac blue color persists for at least fifteen seconds. Tillman's reagent is prepared so that 20 mg. of the dye is contained in 100 c.c. of the dye solution. It is then standardized against a standard solution of cevitamic acid. Since it has been determined that 1 mg. of cevitamic acid is the equivalent of 2.116 mg. of Tillman's reagent, result may be easily calculated in terms of the amount of cevitamic acid excreted in twenty-four hours.

STREPTOCOCCI, Hemolytic, Simplified Method for Grouping by Precipitin Reaction, Brown, J. H. J. A. M. A. **111:** 310, 1938.

The culture is grown in 5 c.c. of infusion broth containing 1 per cent of dextrose for from eighteen to twenty-four hours at 37° C. Many strains grow in the form of a sediment at the bottom of the test tube; others need to be centrifuged. All but about 1 c.c. of the supernatant broth is pipetted off and discarded. Two drops of metacresol purple indicator (0.04 gm. dissolved in 60 c.c. of 95 per cent alcohol and then diluted to 100 c.c. with distilled water) are added to the remaining sediment suspension. From a drop bottle 2 per cent hydrochloric acid (about 6 per cent concentrated hydrochloric acid) is added until the indicator turns slightly pink (about pH 3.0). The tube of sediment is heated in a boiling water bath with occasional shaking for fifteen minutes, and then cooled in running cold tap water for ten minutes. From a drop bottle 2 per cent sodium hydroxide is added until the color of the indicator passes through yellow and just begins to darken (about pH 7.5) but should not be noticeably purple. The tube is then centrifuged for about fifteen minutes, and the clear supernatant fluid used for the precipitin test. It has not been necessary to dilute the antigen.

TECHNIQUE OF THE TEST

On the bottom surface of a nearly optically perfect Petri dish (a satisfactory brand of such dishes is known as "Plano") rule 12 mm. squares by means of a wax or diamond pencil. On the abscissa indicate the sera to be used, e.g., A, B, and C. On the ordinate indicate the antigens. Both inside and outside surfaces of the bottom of the Petri dish must be very clean and free from lint, dust, and finger prints, but need not be sterile. Within the appropriate squares and on the inside surface of the bottom of the dish, place one small (2 mm.) platinum loop of antigen and one loop of serum, mixing the serum with the antigen as added, so as to make rather flat hanging drops when the dish is inverted. A platinum loop is specified because some of the cheaper substitutes give off alkali. To avoid carbon particles in the drops, it is essential to burn off the loop thoroughly, preferably after dipping it into water to remove most of the serum each time before flaming. It may be necessary to centrifuge the sera occasionally to free them from any particles of native precipitate. One should be careful not to form a precipitate by introducing a hot loop into the serum or antigen. Into the lid of the Petri dish is placed a disk of moist, but not too wet, white filter paper. With the bottom uppermost the bottom is placed into the lid of the dish.

Reading of Results.—The assembled Petri dish is placed bottom up on the stage of a microscope, and the drops are observed through a 16 mm. objective. The optimum illumination for observing particles of precipitate is secured by closing the diaphragm of the condenser until a small (about 2 mm.) spot of light appears on the moist filter paper beneath the drop. With very little experience there need be no question about the interpretation of results. One soon learns to distinguish particles of foreign matter from the specific precipitate. In

the sera which the author used the result was usually apparent within fifteen minutes, and in one hour at room temperature it was fully developed. The plates were refrigerated overnight as a matter of routine, but this did not prove of any advantage. Seldom have there been any cross reactions within one hour. Sometimes as the precipitate is forming, it assumes a diffuse ground-glass appearance, which may be caused to form clumps by carefully rocking the dish.

Using the technique described, the author has grouped 236 strains of hemolytic streptococci of groups A, B, and C. Of these strains, 76 had been grouped by other investigators by the Lancefield technique, and the author's grouping was entirely in agreement with theirs. A few strains of other serologic groups were also tested and gave no percipitate with serum A, B, or C.

SPUTUM EXAMINATION, Comparison of Direct Smear, Flotation-Concentration and Culture in, Smith, C. R. Am. Rev. Tuberc. **38**: 57, 1938.

A single technique is described for the simultaneous examination of sputum by direct smear, flotation-concentration, and culture.

A comparison is made of the results of simultaneous direct smear, flotation, and culture in the examination of 304 sputum specimens from 146 persons.

Sixty-three persons were positive, 28.5 per cent by direct smear, 69.8 per cent by flotation, and 88.8 per cent by culture.

Ninety-six specimens were positive; 19.7 per cent by direct smear, 62.4 per cent by flotation; and 88.5 per cent by culture.

Culture and flotation are overlapping and supplementary. Each test is capable of demonstrating tubercle bacilli, not detectable by the other. The routine performance of the two tests by a single technique is worth while.

Procedure.—The sputum of three days is collected in an 8 ounce bottle. A "likely particle" is selected with a wide-mouth pipette, and smeared on one end of a glass slide. The remaining sputum is shaken five minutes in a shaking machine, with an equal amount of 0.5 per cent sodium hydroxide. Two cubic centimeters of this preparation are removed and mixed with an equal amount of 2.5 per cent oxalic acid in a centrifuge tube. After incubation at 37° C. for thirty minutes, the acid mixture is centrifuged, and the sediment planted on two tubes of Petragnani's medium.

The sputum-sodium hydroxide mixture remaining in the bottle is subjected to the flotation test. It is incubated at 55° C. for thirty minutes, diluted to 200 c.c., and shaken five minutes with 1 c.c. of xylol. The creamy layer that appears at the top, after standing, is skimmed off with a pipette and spotted on the other end of the glass slide, three to five layers deep.

Direct and flotation smears on the same slide are fixed, stained by heating with carbol-fuchsin, decolorized, and counterstained with 1 per cent picric acid.

All solutions and glassware used must be sterile.

FUNGI, Pathogenic, Production of Surface Growth of, on Culture Mediums, Arch. Dermat. & Syph. **38**: 32, 1938.

Phosphate, lactate, and a combination of the two increase the surface growth of certain pathogenic fungi. With combination or combinations will act depends on the organism. The base medium consists of 0.25 per cent cysteine and 4 per cent dextrose.

Phosphate, lactate, and the two together produce striking gross morphologic changes in the colonies of certain pathogenic fungi.

It is suggested that substances which result in the change of organisms from a subsurface growth to a surface growth may be of value in bringing them to the surface in cutaneous infections, so that they can be cultured and more easily destroyed by antiseptics.